STUDIES ON SURFACE TAINT BUTTER

by

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C O N T E N T S

STUDIES ON SURFACE TAINT BUTTER

I. INTRODUCTION.

The work herein reported was undertaken jointly by Dairy Research, Science Service, Dominion Department of Agriculture and the Department of Dairying, University of Alberta, to ascertain the cause of surface taint butter and the factors influencing its appearance.

II. HISTORICAL.

Jensen (1891) described a bacterial species, called by him Bacillus foetidus lactis, isolated from cream, which caused a turnip-top or Kohl-rabi odor in milk. He mentions that the organism did not survive long in butter.

Gilruth, of New Zealand (1899) developed a "foetid" odor in butter by inoculating into cream prior to churning a water borne bacterium which he called Bacillus fluorescens liquefaciens. The bacterium gained entrance into commercial butter through the wash water and storage at even 32° F did not inhibit the development of the foetid odor.

Eckles (1900) reported an outbreak of "putrid" butter, unsaleable for table use, made by an Iowa creamery. A large number of gelatin-liquefying organisms were obtained from the affected butter. Of these, four caused an objectionable change in milk. One of the four, Bacterium fluorescens liquefaciens, did not cause the appearance of the defect in butter made from cream in which the organism had grown, while another unnamed species gave a more objectionable reaction. Control measures included a general cleanup, use of a heavier starter

and the rejection of all poor cream supplies.

Conn (1907), mentions that putrid butter was a rarely-seen difficulty but was reported in one or two cases. He cites Jensen's work of 1892, in which putrid butter was attributed to a species of bacterium related to B. putrificus.

Orla-Jensen of Denmark (1910) studied a bacterially-caused, specific defect of butter, the butter rapidly acquiring a peculiar putrid odor that ruined it for table use.

Marker (1919) named a defect of Western Canadian creamery butter "surface taint". Sadler et al (1926) studied surface taint butter from the source which Marker had first noticed. They concluded that post-pasteurization contamination of the cream and of the butter from water supplies and liner-brine was responsible for the defect. A thorough cleanup in the plant stopped the trouble. In experimental work Sadler and Cameron (1926) were unable to duplicate surface taint butter but found that low neutralization of the cream together with a spore-forming, milk digesting aerobe in association with members of the Escherichia-Aerobacter group gave a condition which somewhat resembled surface taint.

Hunziker (1927), quoting Macy, reported that a large coccus in association with a yeast was responsible for surface taint. Again, quoting Macy and also Cordes, Hunziker stated that surface taint butters were high in bacteria, yeasts and molds. This observation was, in the main, sustained by Hood and White (1928), although they noticed some inconsistencies in their counts. In general surface taint butters were found to have a high total bacterial count as well as large numbers of curd decomposing organisms. But, in some factories both low and high bacterial, yeast and mold counts were observed. In some

raw-cream butters lower bacterial counts than in pasteurized surface taint butters were encountered. Surface taint butters were found not to be abnormal in acidity, or curd content, nor could faulty methods of neutralization produce the defect; salt content ranged as high as 2.67%. By inoculating cream with certain water-borne bacteria, Hood and White were able to reproduce the defect.

The spasmodic occurrence of a defect in New South Wales butter, described as "disagreeable aroma" by Brown (1928) suggests that he was dealing with surface taint. He concluded that the odor was one of decomposing nitrogenous matter such as casein or albumen, but that such decomposition took place in crevices of various wooden pieces of equipment with which the butter came in contact, or from curdy material extruded from glands and packing of storage vats. During the manufacturing process this material became incorporated in minute particles in the butter and there, apparently by enzymatic action, continued to produce the volatile aroma. Replacing of old, fat-saturated, wooden equipment and thorough general cleanup of the other equipment caused the trouble to disappear. He states that the odor was not inducible in raw cream butter.

Shutt (1929) claimed to be able to reproduce the defect by inoculating cream with Pseudomonas fluorescens and then churning the cream. Entrance of the organism into commercial butter was considered to have occurred through the wash water, with low acidity as an aiding factor.

Sutton (1929) pointed out two characteristics of rabbito butter. One - its rapid appearance in apparently good

butter and two - the seeming lack of correlation between the bacterial flora (quantitative and qualitative) and the defect.

Derby and Hammer (1931), unable to substantiate Shutt's claim regarding Ps. fluorescens, isolated from surface taint butter an organism capable of causing the defect when allowed to grow in cream prior to churning. They described the organism, naming it Achromobacter putrefaciens. They, along with Hood and White, report the production of surface taint by other unidentified bacteria. Derby and Hammer found that there was a higher bacterial count for the outside of the sample of surface taint butter than for the inside of the same sample. They were unable to duplicate the defect by inoculating the finished butter either with Ach. putrefaciens or with other surface taint butter. Since the organism is susceptible to destruction by heat they concluded that there must be sources of the organism in the plant. For the control of surface taint Derby and Hammer stress cleanliness of equipment, proper workmanship and low storage temperatures of the butter. Variations in surface taint butters were thought to be due to different causal organisms.

Stocker (1931-32, as cited by Herreid et al) reported that organisms belonging to the Bacterium fluorescens group produced in butter an ester-like raspberry odor which passed through a transitional stage of cheesiness to putridness.

Lock (1931), as quoted by Cullity and Griffin, concluded that when plants were careless in cleanup, as during flush seasons, rabbito was more prone to appear. Improper preparation of lumpy cream for pasteurization, badly worked

butter and very poor water supplies were also cited as inducing factors. He regarded Ps. fluorescens as a possible cause of rabbito butter.

Inasmuch as the term "cheesy" is being used in the United States to include the defect caused by Ach. putrefaciens the extensive work of Herreid, Macy and Combs (1934) on the cheese-like flavors of unsalted butter should be mentioned. Neither bacterial types found in aseptically-drawn milk nor the enzyme galactase in cow's milk were able to induce cheesiness in unsalted butter. From raw-cream, pure cultures of various bacteria (predominantly gram-negative rods) were isolated, which were able to induce cheesiness in unsalted butter, although not consistently. While naturally-mixed cultures (also predominantly gram-negative rods) were most consistent in producing cheesiness, artificially-mixed cultures produced only flavors suggesting the cheesiness produced by the naturally mixed cultures. Associative action was suggested as the reason for the consistent production of cheesiness by mixed cultures, while pure cultures were inconsistent. Creamery water supplies, contaminated with bacteria capable of producing the cheesy flavors, were thought to infect the butter through the butter wash water. By churning washed cream it was found that the plasma of the cream contained the substrate necessary for the development of cheesy flavors and aromas by the causative organisms.

Loftus Hills, Scharp and Searle (1935), as quoted by Cullity and Griffin, showed that rabbito butter when worked into (1) unsalted sterile butter, (2) sterile butter, 1% salt, (3) sterile butter, 2% salt, caused the development of its odor

characteristics in the inoculated samples. They isolated Ach. putrefaciens from factory water supplies, churns, raw cream and pasteurized cream. The churn was considered the immediate source of contamination of the pasteurized cream. Poor texture was mentioned as rendering the butter more susceptible to development of rabbito.

Randell (1936) is quoted by Jr. Dairy Res. 1937 as follows: "A musty flavor in butter may be due to a species of Achromobacterium".

Sproule and Hamilton (1937) considered the water supply, butter washwater in particular, to be the important avenue of contamination by bacteria capable of causing the defect they called "surface flavour". They advised chlorination at the rate 5 - 10 ppm, or the use of a bacterial filter on the water main, but were unable to obtain defective butter by the use of water from the troubled creameries.

Rice (1937) is quoted by Mattick et al, as follows: "Rabbito in Australian butter is due to the growth of Achromobacterium (occasionally Pseudomonas) and occurs in both unsalted and salted butters".

Dahle and Josephson (1937) found that the introduction of 0.5% to 2% of an aqueous extract of Avenex into cream prior to pasteurization and churning prevented the appearance of surface taint in butter stored at 40-45°F. for 8 weeks. They concluded that the Avenex had a retarding effect on oxidative deterioration.

Cullity and Griffin (1938), by means of pilot churnings in a plant troubled with rabbito, traced the source of contamination to the churn and by the use of a new churn barrel prevented its reappearance. However, later work threw doubt on this conclusion, for rabbito reappeared, showing no consistency as to the butter coming from any one churn. A thorough overhaul of the plant failed to prevent the recurrence of rabbito butter. It was noticed that butter containing rinsings from a certain vat was affected. Chlorination of the water supplies alleviated the condition for some time but later was found to be not regularly effective. A new well was bored and except for a few spasmodic occurrences immediately after the change in water supply this particular factory had no further trouble. Irregular quantitative bacteriological results were obtained on affected butters and isolations from plates failed to duplicate rabbito in experimental churnings. Two water samples yielded Ach. putrefaciens, which was found capable of causing the defect. Salt, curd, fat and moisture analyses yielded no significant results. Mention is made of the working, in lots of a box, or two at a time, of poor butter into good churnings. Such mixed churnings were subsequently graded rabbito. Other churnings made in the same churn after this period also developed rabbito, which seemingly indicated equipment contamination. In one plant more thorough working of the butter sufficed to clear up the trouble. Their conclusions and recommendations were essentially those of Loftus Hills et al.

Totman, McKay and Larsen (1939) in their text on

Butter associate "so called putrid or limburger" flavors with other surface flavors, but feel that the two classes are of different origin. They also associate cheesy flavors with putrid or limburger flavors and conclude that excessive curd in low-grade-cream butter which has been overneutralized may contribute to protein decomposition with later putrid development. To guard against surface and putrid flavors they recommend careful routine plant sanitation, careful neutralization and pasteurization and the use of pure wash water.

Parker (1939), in a report of a conference between technical men of several large creamery operating concerns in and around Chicago, states that surface taint is becoming more prevalent in that area. Cartons, parchment wrappers, water supplies and plant sanitation are mentioned as possible factors in its appearance. He makes the observation that several outbreaks took place in plants using high temperature pasturization of cream. The defect was not noticed in butter packed in tubs, only in prints made with a screw type of machine.

Turgasen (1939), presents data somewhat along the same lines as those of Parker, although he calls the defect "cheesy" and includes "putrid" and "surface taint" butter under this heading. The defect is bacterially produced, with creamery water supplies as the source of the causal organism. Some of the organisms capable of causing the defect were found to be resistant to chlorine treatment, but adequate (?) chlorination of <u>all</u> water supplies was judged effective as a control measure. While most of the affected butters had less than 2.5% salt,

the defect was not inhibited by 4% salt. Adjusting the reaction of the cream to give butters with serum pH's from 5.4 to 7.8 apparently had no influence in controlling the defect.

Claydon (1939), and Claydon and Hammer (1939), by the use of a modification of the Burri smear technique, were able to isolate Ach. putrefaciens from 70.7% of typical putrid butter and from the water supply of one plant. Isolation of the organism became more difficult as the butter aged. Ach. putrefaciens did not readily initiate growth on artificial media, which accounted in part for the difficulty of isolation from affected butter. Incubation of experimental butter for one day at 21°C or for seven days at 5°C produced the defect. Ach. putrefaciens, added in such small amounts to either the cream or the wash water that its subsequent reisolation from the butter was difficult, was found to cause the defect in unsalted butter. The pH of commercial putrid butter varied from 5.8 to 6.8, while a pH of 4.5 prohibited the appearance of the defect in experimental butters. Salt contents of commercial putrid butters ranged from 1.08% to 2.4%. Salt was found to have an inhibitory effect on the production of the defect by Ach. putrefaciens only if thorough working was effected. Five per cent butter culture or 0.5% calcium propionate was sufficient to prohibit the development of the putrid condition by Ach. putrefaciens. Claydon concludes that "Because of its characteristics, action in experimental butter and presence in much commercial butter, Ach. putrefaciens was considered an important cause of the putrid type of cheesiness in commercial butter".

III. DESCRIBING AND NAMING THE DEFECT

The name "surface taint" was first applied to a defect of butter by Dr. C. P. Marker (1919), then Dairy Commissioner for the Province of Alberta. The odor was observed in a shipment of Alberta creamery butter in storage in Vancouver in 1919. Marker noticed the defect only on the surface of the affected butter and he felt, at the time, that "surface taint" was a specific fault of butter and was not to be confused with other surface-odor defects.

Surface taint seems to defy adequate description, the following being some of the terms used by various persons engaged in dairy work: "peculiar sweetish flavour somewhat resembling that of condensed milk", "gutted hog", "turnipy", "sickening", "the limburger defect", "putrid type of cheesiness", "rotten cabbage", "sweaty feet".

To those associated with the present work "sweaty feet" seems to give the closest approximation.

Descriptions of butter defects, suggestive of surface taint, were reported as early as 1891. Because it is not known whether the butters were made from pasteurized or from raw cream it is difficult, in the light of work to follow on pasteurization and surface taint, to determine if these defects were the same as the defect we call surface taint.

It seems probable that the "rabbito" defect of Australian butter and Western Canadian surface taint are identical. In the United States a series of defects is grouped by some under the general heading "cheesiness". Among the defects

included in this term is "putrid butter". The term "putrid" embraces the defect caused by Ach. putrefaciens but whether it is used to include defects other than surface taint is not at all clear at present. Presumably it covers any butter defect suggestive of putrefaction.

Ach. putrefaciens is the only named or described bacterium on which there is agreement in the Australian, Iowan and Albertan work as to defect-producing properties. It is claimed to produce "rabbito", "putrid" and surface taint butter.

In Ontario (Shutt - 1929) and in Australia (Rice - 1931) Pseudomonas fluorescens is claimed to be a cause of surface taint and "rabbito" butter. Derby and Hammer (1931), on the other hand, are in disagreement. Results with this organism will be presented in a following section.

Ach. putrefaciens, when grown in sterile skimmilk, produces a characteristic odor which has not been duplicated by any other organism in over 2500 isolations from well waters and commercial butters in this study. It has been found that this odor is intensified when the milk culture is spread on the fingers and the moisture allowed to evaporate to the point of dryness. No other organism has been encountered in this study with this peculiar property. All other odors have been found to decrease in intensity when spread on the fingers.

For laboratory purposes it has been found desirable to distinguish between the skimmilk culture and the finished butter odors produced by Ach. putrefaciens. Therefore, the odor produced in skimmilk by this organism has been called the

"sweaty-feet" odor and that produced in butter the surface taint odor. Up to the present proof has not been forthcoming that the two arise from the same chemical compound. That they are likely to be identical is indicated by the following considerations.

1. The "sweaty-feet" odor is the invariable odor produced by Ach. putrefaciens in heat-treated whole or skimmilk, cream or butter moisture.

2. It has not been possible to demonstrate fat-splitting by this organism.

3. Non-fatty milk compounds are present in butter.

4. In their relation to acidity, opportunity for oxidation, "clinging" characteristics etc. they appear to be similar.

For these reasons when it has been necessary in this study to use milk cultures and the odor therefrom as a measure of surface-taint-producing ability of an organism or for chemical studies, the assumption is made that the "sweaty-feet" and surface taint substances are identical.

Many practical butter men have identified the odor of milk cultures of Ach. putrefaciens as the surface taint odor from defective butter. Those associated with this investigation have come to regard the odors from these two sources as differing, perhaps because any odor coming from butter is necessarily a mixture of odorous compounds. While studying the effect of pH on the growth of Ach. putrefaciens in skimmilk and also the effect of aeration of a skimmilk culture it was found that the odor may be composed of two types of odorous substances, differ-

ing in chemical behaviour. These two types of odors were considered identical one with the other as well as with the odor of surface taint butter by a number of practical butter men. To those connected with this study the odors were distinct and distinguishable with ease. One was the "sweaty-feet" odor and the other was considered to be typical of putrefaction. It seems probable, therefore, that in practice ordinary putrefactive odors may easily be confused with the typical and essential surface taint odor, especially when they are sought for in experimental solutions and conditions.

IV. PRACTICAL EXPERIENCES WITH SURFACE TAINT BUTTER

In the grading of Canadian butter the practice is made of holding small portions of suspicious samples for 24-48 hours at room temperature, at the end of which time an odor-test for surface taint is made. Experienced graders seem to be able to tell by taste that a churning of butter will, under proper temperature and time conditions, develop surface taint. Since its appearance in 1919 there have come to be recognized as fairly well established facts certain noteworthy characteristics of surface taint butter, such as:

1. Its sudden appearance shortly after the general introduction of pasteurization and neutralization.

2. Its sporadic appearance both as to time and place.

3. Butter made from the best cream is usually involved. It has been seen much less frequently in second grade churnings.

4. No authentic record has come to our notice of an occurrence of surface taint in raw cream butter.

5. If it occurs in one part of a box it is in all parts of the box; similarly all boxes of a churning are likewise affected.

V. EXTENT OF SURFACE TAINT BUTTER

Hood and White (1928) state that in 1927, 124 churnings of commercial butter were graded surface taint, with 40 coming from Saskatchewan, 71 from Alberta, 6 from Manitoba, 6 from Ontario and 1 from Quebec. Fifty-one factories were involved, in rough proportion (by provinces) to the number of churnings contributed.

Derby and Hammer citing MacKay, report that in 1928 1.45% to 3.12% of the total make for the different Prairie Provinces was graded surface taint, while the corresponding figures for 1929 were 1.41% to 2.52%. In 1938, 0.4% (or 100,000 lbs.) of the graded make of creamery butter for Alberta was officially graded surface taint. (Report of the Dairy Commissioner for Alberta 1939). Approximately 24,000 lbs. of Alberta creamery butter were officially graded surface taint in 1939. (Report of Calgary Grading Centre, 1940).

VI. BACTERIA IN BUTTER

Since butter is produced from materials known to contain varying numbers of bacteria it is not surprising to know that bacteria become incorporated in butter and their presence there has never been questioned. However, because of the conditions of air, nutrients and water in butter, it becomes questionable whether extensive growth and multiplication of bacteria is possible in the interior of well worked butter, and since surface taint has been attributed by various workers to the growth of bacteria in butter it becomes of interest to examine the

possibilities for such a phenomenon.

Conn (1907) on the basis of the plate count stated that bacteria declined in numbers as the butter aged.

Sayer, Rahn and Farrand (1908) found a decrease in numbers of bacteria in commercial butter in 3 months. They suggest that bacteria might cause butter deterioration without multiplication taking place.

Rahn, Brown and Smith (1909) concluded that salted commercial butter kept better than unsalted commercial butter and that all commercial butters sustained an increase in amide nitrogen, with the poorer butters showing the larger increases.

Rogers, Berg and Potteiger (1913) discounted the work of Rahn et al on the basis of faulty techniques. By improving their technique these workers found no evidence of an increase in soluble nitrogen in butter on long standing at 0°F, even when conditions of manufacture were most favorable for such changes. No simple or obvious relation between the values for soluble nitrogen and the butter score was found. "Bacterial enzym" produced measurable proteolysis as judged by soluble nitrogen determinations.

Brown and Peiser (1916) claimed that about 30% of the bacteria in ripened cream failed to grow after the mechanical agitation in the churn and that 50% of the bacteria in the unsalted butter are removed by the washing and salting processes.

Kildee (1917) considered that deterioration of butter stored at 10°F did not bear a close relation to bacterial

content and that bacteria were not the immediate cause of such deterioration. Unsalted butter was found to keep at 10°F. better than salted butter. This result is at variance with other reports on the effect of salt.

Washburn and Dahlberg (1917) stored salted and unsalted commercial butters at -15°F. Bacterial numbers in the unsalted butter decreased more rapidly at this temperature than they did in the salted butter. Little if any relationship existed between bacterial numbers, acidity and the score of the butters. They found an increase in numbers of bacteria at higher temperatures (58°F.).

Bouska and Brown (1921) as quoted by Grimes (1923) suggested that the deterioration of butter is mainly the result of physical or biochemical causes and that an indirect part may be played by micro-organisms.

Grimes (1923) concluded, on the basis of the plate count of commercial sized churnings, that about 1% of bacteria in ripened cream and about 20% of bacteria in pasteurized cream were retained in the butter. No evidence could be found that either the enzymes produced during the growth of the micro-organisms or their disintegration products affected the keeping quality of butter in cold storage.

Shutt (1924) suggested that efficient pasteurization and freedom from recontamination were the important factors in controlling the keeping quality of commercial butter stored for six months at 10°F.

Spitzer, Parfitt, Manhart and Epple (1927), by measuring soluble nitrogen fractions concluded that the quality of butter decreased in proportion to protein hydrolysis, and proteolysis progressed at a greater rate in the presence of proteolytic organisms. They also state that although salting inhibited the growth of micro-organisms, such treatment did not retard protein hydrolysis. The butter used was made in lots of about 10 lbs., but no details as to method of churning or working are given.

Rahn and Boysen (1928), using microscopic methods, counted and measured water droplets in commercial butter and obtained a frequency table for the distribution of the moisture. They concluded that even in sour-cream butter there are over 100 droplets per bacterium and in sweet - cream butter about 80% of the moisture remains sterile. To account for their finding that more acid was produced in butter than could be calculated for, they assumed that there was a slow diffusion of the acid into sterile droplets. They believe that washing removes casein and lactose from the outside of butter granule aggregates and since the small inside buttermilk droplets are, in the main, sterile a decrease in acid production is found in well-washed butter, for the infected large droplets are almost pure water. Salt, by attracting water, tends to desiccate bacteria. The high brine concentration also inhibits bacterial fermentation.

Macy and Richie (1929) claimed that, as a group, commercial butters with low yeast and mold counts kept better

at -5° to -10°F than butters with such counts high.

Ruehle (1930) concluded that the metallic flavour of commercial butter may be produced by metals, bacteria or amino acids. He listed a variety of bacteria, yeasts and molds which were encountered in butter after storage, but failed to find any definite relationship between numbers or types and specific off-flavours.

Knudsen and Jensen (1930), as quoted by Long and Hammer (1939), found that bacterial activity in unsalted butter decreased as the working was increased. They assumed that working tended to decrease the size of the water droplets and thus reduced the nutrients available for bacterial uses.

Orla-Jensen (1931) states that "in sweet cream butter the bacterial count increases during the first few days, after which it decreases".

Derby and Hammer (1931) reported that "the excessive numbers" of bacteria found in surface taint butter "suggest that considerable growth must have occurred because such high counts would not be expected in butter, regardless of the quality of raw material or the manufacturing methods".

Macy, Combs and Coulter (1932) found that the bacterial counts of unsalted commercial butter stored at 35°F. reached a maximum in about ten days and then fell off gradually, but not sufficiently to bring the counts below the original value. The majority of the samples of salted butter stored at the above temperature sustained reduced bacterial counts which fell

as the length of the storage time increased.

Hammer and Yale (1932) found that in ten days at about 7°C. Escherichia species did not grow in salted, while some Escherichia and Aerobacter species sometimes grew in the unsalted butter. In ten days at about 18°C. species of both genera grew in salted as well as unsalted butter, but the Aerobacter species grew more rapidly and reached higher numbers than did the Escherichia species. The butter used in this work was churned in glass jars and worked with wooden paddles.

Arup and Gilmour (1933), as cited by Hammer (1938) state that butter (presumably commercial) stored at -7°C. for six months sustained no increase in yeasts, molds or bacteria and also no considerable reduction. At -2°, -6°, and -12°C. growth stopped and bacterial counts decreased.

Collins and Hammer (1933) studied the migration of bacteria through unsalted butter and found that this phenomenon, while uncommon, was more noticeable in poorly worked than in well worked butters, presumably because of water channels in the poorly worked butter. Extensive migration was possible in the water which collected between the wall of the container and the butter.

Herreid et al (1934) obtained from 200 to 20,000 times increases in bacterial numbers in experimental butters stored at 10°C. for 14 days.

Grimes and Hennerty (1934) stored commercial butter at 15°F for 56-252 days and found a noticeable increase in yeasts, a decrease in numbers of bacteria and a slight increase in acidity.

Loftus Hills et al (1934) found storage of samples of commercial butter at 12°F for 3 months to effect only slight changes in bacterial numbers. Neither bacterial, yeast nor mold counts revealed a consistent correlation with keeping quality. They suggest that enzyme activity was not an important factor in controlling deterioration, but state that acidity and copper content appear to be most important.

Kellerman (Jr. Dairy Res. 1937), compared judging and bacterial analyses of butter and obtained an agreement in 72% of 265 tests.

Olson (1937), while observing the effect of wash water filtration on butter quality, found both salted and unsalted butter (churned in glass jars and hand-worked in granite pans) stored at 0, 5, 15, and 21°C for 56, 28, 14 and 7 days, respectively, sustained variable increases and decreases in bacterial contents as shown by the plate count. Lower storage temperatures, coupled with longer storage time, gave a larger proportion of decreased counts.

Flake and Parfitt (1937) contend that high lipolytic counts of commercial butter stored at 15.5°C for 10 days had a tendency to be associated with poor keeping quality. Samples developing a putrid flavor were most marked in this respect.

Jacobsen (1937) stated that "The increases in total numbers of bacteria and flavor deterioration in unsalted, non-culture butter were much more extensive than in the unsalted, culture butter held under similar temperature conditions. The growth of bacteria at 21°C was apparently not as much of a

factor in flavour deterioration as growth in unsalted butter at lower temperatures. The more extensive development of lipolytic and proteolytic bacteria at the lower temperatures than at 21°C was indicated as the reason for this condition". This work concerns commercial butter and the publication contains a splendid review of this subject.

Jacobsen (1938) in a study of bacterial content and keeping quality of butter (of unmentioned source) after removal from storage found that -25°C effected marked decreases in total counts, the reduction being more pronounced in unsalted butter. No correlation existed between proteolytic or lipolytic counts and development of flavour defects. The bacterial counts increased more rapidly and flavour deterioration was more rapid in unsalted butter held at room temperature (21°C) after storage at -25°C than in the fresh butter held 7 days at room temperature. The bacterial counts did not change significantly in salted butter held 7 days at room temperature subsequent to storage at -25°C. Neither lipolytic nor proteolytic bacteria were detected in the salted butter which had been held 7 days at room temperature subsequent to storage at -25°C.

Flake and Parfitt (1938) found a limited relation between the microscopic picture and keeping qualities of salted, sour-cream commercial butter stored 10 days at 15.5°C. They did, however, notice a marked relation between high proteolytic counts on T.G.S. agar and poor keeping quality and also between high lipolytic counts on tributyrin medium and keeping quality.

Long and Hammer (1938) discuss the effect of working on the growth of bacteria in butter. At 21°C and at 5°C the growth of various organisms in unsalted butter was most rapid in the poorly worked samples. The time taken for specific bacterial defects (obtained by inoculating the churning cream with pure cultures) to appear was influenced by the degree of working, the thoroughly worked butter taking a longer time to develop the defect than the well-worked. Poorly worked cultured-butter reached a lower pH than the same butter well-worked. The rate of lowering of the pH was also faster in the poorly worked sample and they conclude that the finer dispersion of moisture droplets in well-worked butter decreases the food supply in infected droplets, so retarding spoilage.

Mrozek (1939) carried out storage experiments on 186 commercial butters in 2kg. pats and in 50kg. casks. Keeping quality was very poor and only one butter was of marketable quality after 6 months in a cask at -6 to -8°C. There was a tendency for butters with high bacterial and yeast counts to spoil more quickly than butters of better bacteriological quality.

Totman et al (1939) consider that peptonizing bacteria are undesirable in butter in that they tend to increase butterfat losses during churning, but that they may not necessairly lower butter flavor and quality score by their presence.

In evaluating the literature on the growth of

bacteria in butter there are several important points to be considered, such as:

1. The method used in enumerating the bacteria or in measuring growth increases.

2. The source of the butter. Was the butter used in making the observations obtained from large commercial-sized churnings, or was it made in small lots with laboratory equipment and methods?

3. The state of working and moisture incorporation.

4. Theoretical concepts of bacterial growth requirements.

Most, if not all, of the results on the counting of bacteria in butter have been obtained by the use of the petri plate method, the variability of which has been widely studied and whose accuracy is questioned. In the light of this knowledge it is difficult to make a satisfactory interpretation of some of the data presented in the literature. Such factors as the effect of storage on the break-up of bacterial clumps, the inherent errors of the plate count method itself, and the possibility that space in small water droplets may not be sufficient for active bacterial growth must be taken into account.

From the review presented it will be seen that in practically every instance where bacteria have been shown to increase greatly in butter the butter was made in small lots on a laboratory scale. From our experience in the churning and the working of butter on a small scale we realize that it is well-nigh impossible to get butter of the same texture and moisture inclusion as is to be found in well-worked commercial butters.

We believe that, because of the small volume of butter involved and the relatively large surfaces presented (in comparison with the volume), it is difficult to get dry surfaces on our experimental butter and we caution too rigid interpretation of results based on such butters.

Up until about 1920 commercial Alberta butters were of a much more open texture than those of today. Because of this, conditions more favourable for growth of bacteria in butter are conceivable since the percentage of infected moisture would be higher and the available nutrients would not be so finely dispersed. In this connection it is well to stress the growth of bacteria in and not on butter, for if there is moisture on the surface of butter there are likely to be sufficient nutrients in this moisture to support bacterial growth. However, conditions inside a block of butter present a somewhat different picture. Inside butter, air, moisture and available nutrients have a different distribution than they have at the surface. Rahn and Boysen (1928) consider butter fat to be almost impermiable to the diffusion from moisture droplet to moisture droplet of materials dissolved in the droplet. Since the large majority of moisture droplets are sterile the nutrients in the infected droplets would soon be exhausted and the bacteria would be unable to increase to any great extent.

The effect of salt and of low temperatures can possibly be explained on the basis of actual killing of bacteria as well as on the basis of inhibition of growth.

If bacteria are able to grow in butter and produce

a defect it would be logical to expect that there would be a close correlation between numbers of bacteria and butter score. This, however, has not proven to be the case, as can be seen from the history. Moreover, if bacteria are able to grow in butter and produce defects why is it that so little of the total make is affected, when the presence of undesirable bacteria can be demonstrated to be present in normal butter?

For these reasons it becomes questionable whether extensive growth and multiplication of bacteria is possible in the interior of well-worked butter.

From the above discussion and from the historical review presented, the following facts stand out:

1. There is no agreement as to the growth of bacteria in butter.

2. Enzymes are of questionable importance in the spoilage of butter.

3. There is no correlation between keeping quality and bacterial content of fresh butter.

4. There is no correlation between score of butter and bacterial content.

5. Salt, in general, has an inhibitory effect on butter spoilage.

6. Well-worked butter has better keeping qualities than poorly worked butter.

On the basis of the above history and discussion and on work to follow we feel that the secret of the production of surface taint may not be found in the growth of organisms in butter and that other possibilities deserve scrutiny as well.

VII. A STUDY OF SURFACE TAINT BUTTERS

1. Sources.

Samples of surface taint butter were taken in sterile 4 oz. screw-top jars with sterile spatulas. Dairy Produce Grading Centres at Edmonton, Calgary, Regina, Winnipeg and Vancouver co-operated by sending samples of surface taint butter, which often arrived in a softened condition and before sampling for chemical and bacteriological analyses they were hardened at 10-15°C. Portions for the chemical and bacteriological analyses were removed with a sterile spatula and the portion for the bacteriological work placed in a sterile jar.

2. Chemical Analyses.

Analyses were applied to 40 surface taint butters and to 16 normal butters as follows:

1. Kohman test for moisture, salt and curd.
2. pH - determined electrometrically*.

Referring to Tables I, II, and III, it will be seen that the values for moisture and salt for surface taint butters fell within the same range as those for normal commercial Alberta butters. Through an oversight in the operation of that portion of the test which yielded values for the curd content such values for surface taint butters appear to be lower than for normal butters. The pH extremes for surface taint butters were slightly lower than those for normal butters.

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* The author is indebted to the National Research Council for the loan of the potentiometer and equipment with which (except in cases noted) pH and E_h determinations were made.

3. Bacteriological Analyses.

Thirty-seven surface taint butters along with 26 normal butters were examined as follows:

1. Total plate count on Tryptone-glucose-meat extract - 2% skimmilk agar (hereafter called T.G.S. agar) incubated at 10-15°C for 4 days. Sometimes two dilutions, one plate each, were poured, while in most cases three dilutions were used.

2. Proteolytic count. From the above plates all colonies surrounded by a clear area were considered proteolytic and reported as such. We realize that this involves some error due to the clearing action on milk solids of lactic acid organisms and as will be shown later, the inability of the operator to consistently recognize proteolytic surface colonies and the total inability to recognize proteolytic subsurface colonies.

3. Total count on nutrient gelatin, incubated at 10-15°C for 4 and 5 days.

4. Proteolytic count on gelatin. From the gelatin plates colonies showing frank liquefaction as well as colonies which appeared to be sunken in a crater when observed by oblique light were counted and reported as "gelatin proteolytics".

Technique. Difco materials, with the exception of the skimmilk powder, were used to prepare the media. The pH, checked colorimetrically and occasionally electrometrically, fell between pH 6.8 -7.0.

The butter was carefully melted in a 4 oz. screw-top jar in a water bath at 40-45°C. One cc of the mixed butter fat, curd and moisture was drawn into a warmed sterile pipette and transferred to a warm dilution blank and the dilution water

sucked back and blown out several times to ensure complete emptying of the pipette.

Counting was done with the aid of a Quebec Colony Counter and a hand tally. Where the number of colonies per plate exceeded 300-400 a sector only was counted. All counts are reported on a basis of 1 cc of mixed butter, curd and moisture.

Referring to Tables I, II, IV, V, it appears that surface taint butters do not differ to any marked degree from normal commercial butters in:

1. Total bacterial counts on either T.G.S. agar or nutrient gelatin. On T.G.S. agar 54% and on nutrient gelatin 60% of the counts of the surface taint butters did not exceed 500,000 per cc; the values for normal commercial butter were 89% and 81% respectively.

2. Proteolytic counts on either of the two media. On T.G.S. agar 92% and on nutrient gelatin 57% of the counts of surface taint butters did not exceed 100,000 per cc; the values for normal commercial butters were 92% and 81% respectively.

From Table VI it is seen that all counts of surface taint and normal butters fell, roughly, within the same ranges. Any differences probably are outside the range of reliability of the plate count method, making the evaluation of all such data somewhat uncertain.

From the quantitative plates poured all of the colonies, or if too many, representative types which were surrounded by a clear area and so considered proteolytic were picked into sterile litmus milk. In addition to the "proteolytic" colonies other bacterial types were picked and saved for study.

TABLE I

Surface Taint Butters. Chemical and bacteriological analyses; Regrading data.

Creamery	Sample No.	Moisture	Salt	Curd	pH	Total Count T.C.S.	Total Count Gelatin	Proteolytic Count T.C.S.	Proteolytic Count Gelatin	Time interval in storage (Mo.)	Regrade
A	1	15	1.02	0.72	5.96	>5,000,000	>5,000,000	No clearing	Many liquifiers	5	Rancid
B	2	15.5	1.72	0.23	6.72	910,000	1,000,000	0	360,000	5	S.T.?
C	3	15.2	1.68	0.11	6.08	224,000	700	43,000	<100	5	S.T.
H	4	16.3	1.16	0.54	-	-	-	-	-	7	Rotten & musty.
J	5	14.3	1.22	0.41	5.3	15,300	9,000	< 100	2,000	6	Sour - yeasty.
J	6	15.2	1.46	0.28	5.37	259,000	173,000	5,000	10,000	5½	Rancid,etc
P	7	15.4	1.66	0.13	5.6	51,000	Spr.	13,000	Spr.	7	Clean
Q	8	-	-	-	4.7	700,000	10,000	0	0	7½	-
R	9	14.0	0.87	-	6.50	2,270,000	3,150,000	130,000	2,390,000	7½	Fruity
R	10	14.4	1.15	-	6.42	103,000	1,040,000	8,000	750,000	7½	Dirty and moldy.
R	11	14.9	1.44	-	6.3	30,000	10,000	2,000	3,000	7	Rancid
R	12	15.1	1.18	-	6.35	10,000	16,000	2,000	2,000	7	Rancid
R	13	16.6	1.14	0.70	6.55	9,000	20,000	2,000	0	6½	Rancid Dirty
R	14	15.5	1.10	0.64	6.35	81,000	218,000	39,000	185,000	6½	Dirty
R	15	15.4	1.04	0.66	6.11	< 1,000	11,000	<1,000	2,000	6½	Dirty
R	16	15.6	1.39	0.39	6.3	< 1,000	42,000	< 1,000	0	6½	Clean
R	17	15.5	1.26	0.49	6.7	22,000	26,000	1,000	8,000	6½	Dirty
R	18	16.2	1.02	0.68	6.17	16,300	300	1,700	300	4½	Clean
R	19	16.2	1.34	0.53	6.21	600	100	100	100	4½	Clean
R	20	15.0	1.37	0.38	5.97	800	100	< 1,000	< 1,000	4½	Clean
S	21	15.0	0.46	1.23	6.43	5,000,000	5,960,000	< 10,000	3,360,000	5	Clean
U	22	15.5	1.70	0.09	-	153,000	148,000	30,000	<10,000	7	Rancid
W	23	15.7	1.68	0.12	-	-	-	-	-	3½	S.T.
Z	24	14.9	1.40	0.30	6.95	450,000	430,000	4,000	110,000	6	Clean
Z	25	16.7	1.35	0.54	6.42	372,000	2,190,000	0	430,000	5½	Rancid
Z	26	15.5	1.72	0.12	6.66	333,000	279,000	1,000	22,000	5½	Sour yeasty
Z	27	14.2	0.82	0.80	6.14	>3,000,000	>3,000,000			5½	Sour
Z	28	15.3	1.72	0.02	6.09	>3,000,000	6,580,000		310,000	5½	Clean
Z	29	15.3	1.69	0.06	6.37	3,300,000	1,150,000	30,000	180,000	5½	Clean
Z	30	15.3	1.64	0.10	6.27	>3,000,000	4,770,000	90,000	140,000	5½	S.T.
Z	31	15.8	1.14	0.53	6.09	>3,000,000	Liquified	Cleared	-	5½	Sour yeasty
Aa	32	15.2	1.38	0.37	6.24	6,800,000	49,000	Cleared	12,000	4½	S.T.
Ac	33	-	-	-	4.8	200,000	147,000	6,000	6,000	Sample used up in analyses.	
Ac	34	15.8	1.46	0.32	6.5	3,840,000	Liquified	3,840,000		Sample used up in analyses.	
Ac	35	15.6	1.75	0.07	6.28	>3,000,000	4,200,000	No clearing	930,000	5½	Sour
Ac	36	15.2	1.45	0.33	6.20	>3,000,000	2,300,000	No clearing	239,000	5½	Rancid
Af	37	15.6	0.96	0.78	6.96	1,270,000	310,000	250,000	80,000	4	Sweetish
Af	38	15.9	0.96	0.83	6.93	263,000	214,000	36,000	62,000	4	S.T.
Ag	39	14.8	1.56	0.17	6.26	90,000	48,000	5,000	8,500	5	Oxidized
H	40	15.0	1.70	0.29	6.41	1,050,000	105,000	>100,000	35,000	4½	Clean
	41	-	-	-	-	-	-	-	-	1½	Clean
	42	-	-	-	-	-	-	-	-	1½	S.T.

TABLE II

Normal Commercial Butters. Chemical and Bacteriological Analyses.

Creamery	Sample No.	Moisture	Salt	Curd	pH	Total Count T.C.S.	Total Count Gelatin	Proteolytic Count T.C.S.	Proteolytic Count Gelatin
	1					> 3,000,000	> 3,000,000		
	2					> 3,000,000	2,420,000		1,700,000
	3					5,000	1,000	1,000	< 1,000
	4					2,000	6,000	< 1,000	6,000
	5					63,000	330,000	< 1,000	40,000
	6					37,000	300,000	1,000	11,000
	7					50,000	2,000	2,000	1,000
	8					1,000	2,000	< 1,000	< 1,000
	9					17,000	5,000	5,000	2,000
	10					1,200,000	1,720,000	< 10,000	960,000
	11					2,700	6,800	400	1,200
	12					10,500	42,000	100	5,000
	13					400	500	< 100	300
	14					2,200	1,200	100	100
	15					20,000	15,200	4,000	5,600
	16					19,000	2,500	6,000	900
	17					7,100	5,300	1,500	200
	18					2,500	1,400	1,100	100
	19					4,900	1,000	1,000	300
	20					155,000	84,000	12,000	44,000
H	21	15.4	1.20	0.48	-	25,000	liquified	5,000	
H	22	13.0	1.24	0.31	6.76	62,000	50,000	16,000	8,000
H	23	12.8	1.30	0.30	6.60	29,000	< 1,000	6,000	< 1,000
H	24	13.7	1.20	0.30	7.30	280,000	< 1,000	6,000	< 1,000
H	25	13.95	1.30	0.23	6.60	215,000	liquified	45,000	
H	26					1,300	20,000	200	20,000
F	27	15.4	1.80	0.10?	6.85				
F	28	15.3	0.00	1.65	6.7				
F	29	13.6	0.00	1.46	6.7				
Q	30	13.9	1.9	0.57	6.43				
Q	31	15.4	1.98	0.66	6.88				
R	32	15.2	1.68	0.08?	6.84				
R	33	15.2	1.64	0.16	6.6				
R	34	13.5	1.55	0.17	6.38				
S	35	15.8	0.00	1.65	6.85				
S	36	15.7	0.00	1.62	6.85				

These skimmilk cultures were stored and incubated at 10-15°C.

Five cultures suspected of being capable of producing surface taint and identified as being Ach. putrefaciens or variants thereof were isolated from 2 surface taint samples, Nos. 9 and 26. These butters came from creameries which had had serious trouble with the defect. Four such cultures were isolated from 3 normal butters, Nos. 11, 13, and 15. From the quantitative data it would appear that there was no obvious relation between the presence of Ach. putrefaciens or variants and numbers of bacteria.

The method used in searching for surface taint producing organisms is admittedly arbitrary, involving previously discussed assumptions. Conditions which were favorable for the growth of such organisms as Ach. putrefaciens but which supressed the growth of other forms were sought but were not forthcoming.

TABLE III

Chemical Composition Ranges

	Surface Taint Butter	Normal Butters
Moisture	14.0% - 16.6%	12.8% - 15.8%
Salt	0% - 1.75%	0% - 1.98%
Curd	0.02% - 1.23%	0.08% - 1.65%
pH	4.7 - 6.7	6.43 - 7.3

TABLE IV

Total count distribution for surface taint and normal butters

Range	Frequencies			
	Surface Taint Butter		Normal Butter	
	T.G.S. Agar	Nutrient Gelatin	T.G.S. Agar	Nutrient Gelatin
0- 500,000	20	22	23	21
500,000-1,000,000	2	1		
1,000,000-1,500,000	2	1	1	
1,500,000-	0	1		1
2,000,000-	1	2		1
2,500,000-	0	0		
3,000,000-	1	1	2*	1*
3,500,000-	2	0		2 liquefied
4,000,000-	0	1		
4,500,000-	6	1		
5,000,000-	1	1		
5,500,000-	1	1		
6,000,000	1	2		

* These figures are for counts > 3,000,000.

TABLE V

Proteolytic count distribution for surface taint and normal butters

Range	Frequencies: Surface Taint Butters T.G.S. Agar	Surface Taint Butters Nutrient Gelatin	Normal Butters T.G.S. Agar	Normal Butters Nutrient Gelatin
0- 100,000	34	21	24	21
100,000- 200,000	1	4		
200,000	1	1		
300,000		2		
400,000				
500,000				1*
600,000				
700,000		1		
800,000				
900,000		1		
1,000,000				
1,000,000-				1
2,000,000-5,000,000	1	3		
		2 liquefied 1 spr.	2 were unaccountable	

*This is for range 500,000-1,000,000

TABLE VI

Plate count ranges for normal and surface taint butters

	Total		Proteolytic	
	T.G.S.	Gelatin	T.G.S.	Gelatin
Surface Taint Butters	6,800,000-600	6,580,000-100	3,840,000-100	3,360,000-100
Normal Butters	>3,000,000-400	>3000,000-500	45,000-<100	1,700,000-100

Storage of Commercial Surface Taint Butter

Thirty-nine samples of commercial surface taint butters were placed, upon receipt, in an ice-cream cabinet adjusted to -20.5°C to -12.0°C (-5°F to 10°F) and stored for periods varying from 1.5 to 7.5 months. They were then brought to room temperature and graded by an official butter grader. Of these butters 7 were graded "surface taint"; 21 had defects (which might possibly have masked the surface taint, were it present) other than surface taint; 11 were regraded clean.

TABLE VII

Storage and Surface Taint Retention

Time in Storage	No.	S. T.	Not Surface Taint	
			Dirty	Clean
1½	2	1	0	1
2	0	0	0	0
2½	0	0	0	0
3	0	0	0	0
3½	1	1	0	0
4	2	1	1	0
4½	5	1	0	4
5	5	2	2	1
5½	10	1	7	2
6	2	0	1	1
6½	5	0	4	1
7	5	0	4	1
7½	2	0	2	0
Total	39	7	21	11

From Table VII it would seem that there was but a limited relation between the length of time of storage and the retention of surface taint. While some of these butters lost their original surface taint character most of the others retained it or had it replaced by equally objectionable defects. It might therefore be concluded that storage at -20°C to -12°C (-5°F to 10°F) cannot be relied upon as a method of improving the grade of surface taint butter.

VIII. WORKED AND UNWORKED BUTTERS

Derby and Hammer (1931) were unable to obtain surface taint in finished normal butter inoculated with defective butter. Loftus Hills et al (1935), on the other hand, report success in obtaining "rabbito" butter by this method. It would seem to us that any method proposed for the control of surface taint must necessarily take into account the possibility of growth of bacteria <u>in</u> butter. It is commonly recognized that the working process has a preserving effect on butter. Suspecting this process to be an important factor in the appearance of surface taint through its control of the growth of bacteria a study of the effect of the working of butter by two methods was made:

1. Three hundred and ten samples of normal commercial butter, melted at 40-45°C in sterile jars were placed at 10-15°C until the fat had solidified above the moisture and curd. With a sterile spatula the moisture was exposed by flipping over a section of the solid fat. The samples were then incubated at

10-15. °C and graded at various intervals.

2. Samples of butter were taken in sterile jars with sterile spatulas at the churn in the following order:

Sample		Identity
A	------	Just after draining of the buttermilk.
B	------	Just after draining of the washwater.
C	------	Finished butter.
D	------	Melted finished butter (treated as in 1 above).

These samples were incubated at 10-15°C as above and were graded at various intervals. Besides grades, bacteriological and pH data were obtained in some of the churnings.

Table IX* reports grading results, pH's and bacterial plate counts on 9 churnings from 5 country creameries. Plate counts and pH were determined within 14 hours after churning. The large bacterial counts, shown in Tables IX and XII, are not surprising in view of the fact that in jars containing the unworked samples much free moisture existed which could support bacterial growth, since no provision was made for refrigeration of the samples. From Table XIII it is found that the range of pH of those samples which were graded surface taint at one time in the observation period was within the range of growth for Ach. putrefaciens. Washing, apparently by removing lactose and bacteria, delayed a drop in pH.

*In this and all subsequent tables S.T. will mean surface taint.

TABLE VIII

Grading Summary of 310 Samples of melted normal butter

S. T.		Questionable S.T.		Not S. T.					
				Dirty		Clean		Not Specified	
No.	%	No.	%	No.	%	No.	%	No.	%
24	7.75	32	10.3	21	6.8	75	24.2	158	51
				254		82%			

Table X contains bacterial plate counts and grading data for 6 churnings from 4 creameries. Counts were made on the day that any one of the set of three butters was graded surface taint. From Tables X and XIV it is seen that the counts of the unworked butters were high in comparison to the finished butters. Extensive growth was possible in the free moisture which was on and below the butter granules of the unworked butter.

From Tables VIII, IX, X, XI, and XV, it is seen that apparently a high proportion of the unworked and melted worked samples of butter was considered at one time in the observation period to have surface taint characteristics. Of interest is the fact that not one of the finished unmelted butters showed this defect.

At first it would seem that many normal butters may contain the agent (chemical or biological) which could give rise to surface taint, were it not for the protective action afforded by the working process. This is indeed a tempting conclusion. However, the observations to follow throw strong suspicion on

TABLE IX

Grading pH and Bacteriological data on samples from 9 churnings.

Dream-ery	Chr. and Sample No.	3	7@	9@	10#	13¢	Day of Chr. pH	Plated on Day of Churning: Total Counts T.G.S.	Total Counts Gelatin	Proteolytic Counts T.G.S.	Proteolytic Counts Gelatin
	1a	fruity					4.55	>30,000,000	liquified	-	-
F8	1b	S.T.&sour					5.75	>30,000,000	liquified	-	-
	1c	clean	fruity	fruity	fruity	fruity	6.85	>30,000,000	liquified	-	-
	2a	cardy dirty					4.9	>30,000,000	liquified	-	-
F8	2b	unclean peculiar					6.35	>30,000,000	liquified	-	-
	2c	clean	very weak	S.T.	fruity	fruity	6.85	>30,000,000	liquified	-	-
	1a	not S.T.					5.05	>30,000,000	liquified	-	-
G8	1b	not S.T.					6.70	>30,000,000	liquified	-	-
	1c	clean	clean	clean	clean	clean	6.84	12,900	600	600	300
	2a	S.T.					6.18	30,000,000	liquified	-	-
G8	2b	S.T.					6.42	30,000,000	liquified	-	-
	2c	clean	clean	clean	clean	clean	6.6	6,900	3,000	<100	200
	3a	doubtful S.T.					6.22	>30,000,000	liquified		-
G8	3b	clean					6.60	>30,000,000	liquified		-
	3c	low grade butter	clean	clean	clean	clean	6.38	3,600	800	800	300
P8	1c		clean	S.T.	question-able S.T.	clean	6.85	1,100	100	300	<100
	2a	probably S.T.rancid					5.78	>30,000,000	liquified		-
P8	2b	-					6.42	>30,000,000	liquified		-
	2c	clean	clean	S.T.	S.T.	atypical S.T.	6.70	spreader	1,800,000		190,000
	3a	S.T.?					5.55	>30,000,000	liquified		-
P8	3b	S.T.					6.42	22,800,000	20,800,000	700,000	7,900,000
	3c	clean	clean	S.T.	Question-able S.T.	atypical S.T.	6.43	580,000	liquified	<10,000	
AB8	1a	-									
	1b	not S.T.					5.35	>30,000,000	liquified		-
	1c	clean	weak S.T.	clean	clean	S.T.	6.60	15,400	21,400	3,600	<100
	2a	S.T.					5.60	>30,000,000	liquified		
AB8	2b	S.T.?					5.95	>30,000,000	liquified		
	2c	clean	clean	clean	clean	S.T.	6.88	7,000	3,900	3,000	400

@ "C" samples - melted in a sterile jar and incubated in the ice box. Graded by H.R.T. and H.W.

#"C" samples - same samples as under@. Graded by W.J.B.

¢ Sterile H20 was added to clean samples on 10th day. Grading by H.R.T. and H.W.

TABLE X

Grading and bacteriological data on samples from 6 churnings.

Creamery	Chr. & Sample Number	(Upon receipt) pH	Total Count (Plated on day when one sample of a churning was graded S.T.) T.G.S.	Total Count Gelatin	Proteolytic Count T.G.S.	Proteolytic Count Gelatin	Grading (Days after churning) 2	3	4	5
	A	4.7	> 30,000,000	910,000	-	280,000				not S.T.
X8	B	4.9	> 30,000,000	8,800,000	-	2,000,000				S.T.
	C		187,000	1,640,000	8,000	300,000				clean
	A	5.25	570,000	520,000	16,000	230,000				S.T.
W8	B	5.7	125,000	103,000	8,000	52,000				S.T.
	C	6.75	800	600	400	600				clean
	A		237,000,000	-	9,000,000	-		S.T.	unclean	clean
W9	B		23,400,000	-	1,000,000	-		clean	clean	clean
	C		2,000	-	< 1,000	-		clean	clean	clean
	1a		58,000,000	-	1,000,000	-	clean	clean		
09	1b		7,000,000	-	1,300,000	-	clean	clean		
	1c		< 1,000	-	< 1,000	-	clean	clean		
	2a		> 200,000,000	-	-	-	clean	fishy		
09	2b		> 200,000,000	-	-	-	clean	S.T. (light)		
	2c		< 1,000	-	-	-		clean		
	A		> 300,000,000		-		clean	clean	clean	clean
E9	B		> 300,000,000		-		"	"	"	S.T.?
	C		14,000		8,000		"	"	"	clean

TABLE XI

Grading Data on worked and unworked samples from 27 churnings

Creamery	Chr.& Sample No.	Grade (Days after Churning) 5	6	7	8	10	11	13
	1a	Clean		off-not S.T.	unclean			dirty
D8	1b	"		S.T.?	clean			rancid
	1c	"		clean	acid clean			clean
	2a	"		fruity				
D8	2b	"		"				
	2c	"		clean				
	3a	"		fruity				
D8	3b	"		rotten				
	3c	"		clean				
	1a							
I8	1b		sour					
	1c		clean					
	2a		metallic					
I8	2b		moldy					
	2c		clean					
	a					S.T.*	putrid**	
N8	b					fruity	clean	
	c					clean	clean	
	a							
M8	b					fruity	fruity	
	c					clean	clean	
	a					fruity	acid	
V8	b					"	musty	
	c					clean	clean	
	a					fruity	papery	
Y8	b					"	sour	
	c					clean	clean	

* Graded by H.W.

** Graded by W.J.B.

TABLE XI (continued)

Grading Data on worked and unworked samples from 27 churnings

Creamery	Chr. & Sample No.	Grade (Days after Churning) 3*	4**
	a	cowy	sour-fruity
V8	b	probably S.T.	rancid
	c	clean	clean
	a	off	rotten apple
J8	b	clean	sour
	c	clean	clean
	a	fruity	sour and cheesy
AE8	b	fruity	sour and cheesy
	c	clean	clean
	a	dirty	sour-fruity
T8	b	clean	sour and cheesy
	c	clean	clean
	a	fishy	fishy
AD8	b	clean	clean
	c	clean	clean

* Graded by H.W.
**Graded by W.J.B.

TABLE XI (continued)

Grading Data on worked and unworked samples from 27 churnings

Creamery	Chr.& Sample No.	Grade [1] (Days after Churning) 2	3	4	5	6
E9	1a	clean	clean	clean	clean	
	1b	"	"	"	S.T.?	
	1c	"	"	"	clean	
	1d	"	"	"	"	
F9	1a	Sample not taken				
	1b	" "	"			
	1c	sl. off	clean			
	1d	clean	no S.T.			
F9	2a	cowy& rancid	S.T.+			
	2b	clean	S.T.+			
	2c	clean	sl.unclean			
	2d	clean	no S.T.			
F9	3a	S.T.?	S.T.+			
	3b	S.T.?	sl. fruity			
	3c	clean	clean			
	3d	clean	no S.T.			
K9	1a		clean	clean	clean	
	1b		"	"	"	
	1c		"	"	"	
	1d		"	"	"	
K9	2a		"	"	"	S.T.?)
	2b		"	"	"	S.T.?)*
	2c		"	"	"	
	2d		"	"	"	
L9	a	clean	"			
	b	S.T.suspic.	green pigment?			
	c	clean	clean			
	d	clean	clean			

1. Graded by H.R.T. and H.W.

* Graded by C.P.M. as being S.T.? but not as such by H.R.T. and H.W.

TABLE XI (continued)

Grading Data on worked and unworked samples from 27 churnings

Creamery	Chr. & Sample No.	Grade [1] (Days after churning) 2	3	4	5	6
	1a	clean	clean			
	1b	"	"			
09	1c	"	"			
	1d	"	"			
	2a	"	fishy			
	2b	"	S.T.(light)			
09	2c	"	clean			
	2d	"	"			
	1a		S.T.	unclean	clean	
	1b		clean	clean	"	
W9	1c		"	"	"	
	1d		"	"	"	
	1a		"	"	"	
	1b		"	"	"	
X9	1c		"	"	"	
	1d		"	"	"	
	1a			"	"	
	1b			"	"	
Z9	1c			"	"	
	1d			"	"	
	2a			"	S.T.?)*	
	2b			"	S.T.?)	
Z9	2c			"	clean	
	2d			"	"	

1 Graded by H.R.T. and H.W.

* Graded by C.P.M. as being S.T.? but not as such by H.R.T. and H.W.

TABLE XII

Bacteriological data summary for 9 churnings plated on day of churning

Samples	Total Count		Proteolytic Count	
	T. G. S.	Gelatin	T. G. S.	Gelatin
A	>30,000,000-	liquified	uncountable-700,000	liquified
B	>30,000,000-22,800,000	" -1,800,000	uncountable 700,000- 100	"-7,900,000
C	>30,000,000-1,100	" -<100		"-200

TABLE XIII

pH data summary for 9 churnings

Samples	Range of pH	Range for S.T. Samples
A	4.55 - 6.22	5.55 - 6.22
B	5.75 - 6.70	5.75 - 6.42
C	6.38 - 6.88	6.43 - 6.88

TABLE XIV

Bacteriological data summary for 6 churnings, plated on the day that one of the set was graded surface taint or questionable surface taint

Samples	Total Counts		Proteolytic Counts	
	T. G. S.	Gelatin	T. G. S.	Gelatin
A	237,000,000-570,000	liquified-910,000	uncountable-16,000	liquified 230,000
B	200,000,000-125,000	liquified-103,000	uncountable-8,000	uncountable 52,000
C	187,000-1,000	liquified-600	8,000-400	300,000- 1,000

TABLE XV

Grading summary - 39 churnings from 21 creameries.

Samples	S. T. and questionable S.T.	Not S. T. Dirty etc.	Not S. T. Clean	Totals
A	12 (32.4)[v]	17 (46.0)	8 (21.6)	37*
B	15 (41.7)	13 (36.1)	8 (22.2)	36*
C	0 (0)	1 (2.5)	38 (97.5)	39
D	6 (27.3)	2 (9.1)	14 (63.6)	22**
Totals	33 (24.6)[r]	33 (24.6)	68 (50.8)	

* Discrepancies due to fact that only 2 samples were obtained from some churnings.
** Not all C samples were melted.
v Per cent of total number of each group.
r Per cent of total number of samples observed.

TABLE XVI

Grading of 12 butters by 3 persons

Butter Sample	Butter Judge 1	2	3
1	S. T.	S. T.	Fruity
2	S. T.	S. T.	questionable S.T.
3	S. T.	S. T.	S. T.
4	S. T.	S. T.	fruity
5	S. T.	S. T.	S. T.
6	oxidized	not S. T.	clean
7	acid	"	acid
8	oxidized	"	questionable S.T.
9	oxidized	"	clean
10	oxidized	"	clean
11	oxidized	"	clean
12	oxidized	"	clean

	S.T.	Not S.T.	S.T.	Not S.T.	S.T.	Not S.T.
12 butters	5	7	5	7	2	10

the validity of such a conclusion. Firstly, three experienced butter men did not agree completely on the grading of 12 samples of melted butter suspected of having surface taint characteristics (See Table XVI). While this is not an indictment of the ability of these three men to recognize surface taint when present in commercial butter, it does throw some suspicion on the ability of an individual to recognize surface taint under experimental conditions such as are imposed upon those working on this subject. Indeed, one of the main problems confronting us is the unsatisfactory criteria we possess for the absolute recognition of the presence of surface taint. As mentioned in a previous section and as will be presented in sections to follow odors which were considered to be typical of surface taint by some were definitely thought to be otherwise by those associated with this work. Secondly, extensive bacteriological work on the worked and unworked samples of butter failed to establish the ubiquity of "sweaty-feet" producing organisms in butters. Cultures suspected of having surface taint producing ability were isolated from 4 unworked samples of 3 churnings of butter from 3 different creameries. These cultures are awaiting identification.

The interpretation placed upon these data is:

1. That putrefactive bacteria of many types grew in the free moisture of the unworked and melted butters.

2. That the odors arising from these butters could easily be mistaken for the surface taint odor by those not well acquainted with putrefactive odors.

3. That the results have a questionable bearing on the surface taint problem.

IX. CREAMERY WATERS IN RELATION TO SURFACE TAINT BUTTER

1. Water in Relation to Butter Quality.

The topic of the relation of the purity of creamery water supply to butter quality has been quite widely studied. The general consensus is that butter washwater may contribute bacteria to butter and so lead to a reduction in quality. It is on this basis that creamerymen endeavor to obtain and maintain a bacteriologically "pure" washwater. This involves the purity of the water as it comes from the main or well and the subsequent care of equipment with which it comes in contact.

The literature is replete with reference to the effect of washwater on butter quality. Gilruth (1899), Eckles (1901), Sadler et al (1926), Hood and White (1928), Shutt (1929), Derby and Hammer (1931), Virtanen (1931), Rumment (1931), Macy et al (1932), Orla-Jensen (1931), Herreid et al (1934), Sproule and Hamilton (1937), Olson (1937), Hammer (1938), Cullity and Griffin (1938), Totman et al (1939), Turgasen (1939), Hammer and Claydon (1939), and others, consider that water may be and in some cases has been shown to be, an important item in determining the quality of the butter produced.

Mrozek and Meetz (1931) are cited by Herreid et al as obtaining no definite correlation between water supply quality, bacteriologically and chemically, and the quality of the butter. This finding seems to be at variance with other reports.

The purity of a water supply as it affects surface taint production seems to us to be of vital importance. The reason for this is not so much that growth in butter of bacteria which are capable of causing the defect takes place, but rather

that through the water supply used in washing plant equipment these bacteria are introduced (in some cases in large numbers) into the equipment and there may grow on residual milk solids to produce the material which subsequently becomes incorporated in the butter and gives us the odor and flavor characteristics of the defect. The churn, since it is one of the most difficult pieces of equipment to maintain in a satisfactory condition of cleanliness and since it comes in very intimate contact with all the butter, would seem to us to be the most serious offender as a source of contamination of the butter with the products of bacterial growth.

This contention is difficult to prove experimentally, but observations in actual practice seem to bear it out. An illustrative case is as follows: Churning '11' of a certain Alberta creamery was officially graded surface taint. The next churning, '12' made from selected cream, was intended for exhibition purposes and therefore extra care was given to the plant equipment prior to processing of the cream into butter. No special precautions as to the use of a different water supply were taken. This churning, as intended, was of exhibition quality. Churning '13', however, was officially graded surface taint. It has been repeatedly shown that the water supply of this creamery is heavily inoculated with Ach. putrefaciens and this organism has been isolated from samples of butter made on these premises. Other churnings following Number '13' were also graded surface taint, although no apparent consistency was evident in its

appearance. We believe that the surface taint churnings made in this plant resulted from the incorporation of bacterial growth products in the butter. In the case of the exhibition churning the equipment was given rigid cleansing and we feel that this sufficed not only to cut down bacterial growth but also to remove accumulated growth products.

2. A Survey of Creamery Water Supplies.

A bacteriological survey of 45 well and 35 tank waters from 37 Alberta creameries was made in an attempt to determine the extent of surface taint producing organisms, since water has been suspected by us and by other workers as being an important source of such bacteria.

Samples, collected in sterile paper-covered screw-cap medicine bottles, were sent to the laboratory in lots of 5 - 12 in insulated cartons without refrigeration. Approximately 1½ days elapsed between the taking and plating of the samples. Plating was done in three dilutions on T.G.S. agar and nutrient gelatin as described in a previous section on butter. From the plates poured colonies were picked into litmus milk and saved for further study.

A study of Tables XVII, XVIII, and XIX shows that total and proteolytic counts on T.G.S. agar varied roughly with the same counts on nutrient gelatin. However, nutrient gelatin counts, both total and proteolytic, seemed in most cases to be slightly higher than corresponding T.G.S. agar counts. This held especially for the proteolytic counts on gelatin.

TABLE XVII

Creamery Waters

Source	Sample	Tank or Well	Total Counts T.G.S.	Total Counts Gelatin	Proteolytic Counts T.G.S.	Proteolytic Counts Gelatin
AJ	1	W	300	800	0	200
	2	W	1,000	6,000	500	5,000
	3	W	312,000	490,000	6,000	340,000
	4	T	610,000	680,000	20,000	650,000
	5	T	312,000	420,000	205,000	120,000
AM	6	W	2,000	25,000	0	10,000
	7	T	5,000	120,000	300	80,000
AI	8	W	170,000	460,000	150,000	440,000
	9	T	65,000	66,000	49,000	56,000
AN	10	W	100	3,000	0	0
	11	West T	600	500	200	200
	12	East T	11,400	7,000	4,300	6,100
Q	13	W	1,000	7,000	0	0
	14	T	2,000	6,000	0	1,000
	15	T	100,000	1,000	10,000	1,000
AD	16	W	90,000	20,000	0	6,000
	17	T	10,000	13,000	0	2,000
AP	18	W	16,000	1,000	0	0
AQ	19	T	510,000	590,000	0	70,000
	20	W	0	20,000	0	10,000
	21	City of Calgary	500	0	0	0
AD	22	T	7,100	7,000	1,000	3,000
T	23	W	1,000	400	1,000	400
	24	T*	1,400	9,100	0	300
AR	25	T*	0	100,000	0	10,000
AK	26	W	170,000	250,000	2,000	100,000
	27	Pump	100	100	0	0
	28	at churn	1,000	200	100	0
AS	29	W	7,000	8,000	500	2,000

*After 2 more days proteolytics were too many to count on T.G.S.

(continued)

[illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

TABLE XVII (continued)

Creamery Waters

Source	Sample	Tank or Well	Total Counts T.G.S.	Total Counts Gelatin	Proteolytic Counts T.G.S.	Proteolytic Counts Gelatin
AE	30	W	4,000	5,500	200	1,000
	31	T	2,000	1,000	0	200
U	32	T	131,000	148,000	1,000	38,000
AT	33	W	10,000	11,600	0	2,800
Z	34	W	70,000	305,000	0	210,000
	35	T	17,900	2,470,000	900	440,000
AU	36	NW	10,000	13,000	0	3,700
	37	SW	5,000	33,000	5,000	16,000
	38	T	9,000	3,000	5,300	600
AV	39	EW	3,000	liquified	0	liquified
	40	2W W's	40,000	680,000	3,000	280,000
	41	T	230,000	960,000	5,000	340,000
AW	42	B.R. W	100	500	0	0
	43	P.h. .W	9,000	3,600	300	700
	44	T	7,000	72,000	0	31,000
X	45	W.#1[1]	9,000	900	0	300
	46	W.#2	10,000	6,000	600	1,000
	47	T-W.#1	170,000	29,000	?	11,000
	48	Mixed T	20,000	6,000	1,000	1,700
K	49	W	0	0	0	0
	50	T	0	1,000	0	0
W	51	W[1]	8,700	13,500	0	500
	52	T[1]	9,000	3,000	0	3,000
AX	53	W	0	0	0	0
	54	T	3,600	1,500	3,000	1,000
E	55	W[2]	3,800	3,000	200	900
	56	T	32,000	2,000	200	800
AY	57	W	0	0	0	0
	58	T[3]	60,000	1,300	300	500
L	59	W	16,000	13,000	0	1,200
	60	T	7,000	4,000	1,700	2,000

1 T.G.S. proteolytic showed big increase in 2 more days.
2 In 1 day extra T.G.S. total - 5,300 and Proteolytic 1,100.
3 Pin point colonies were slightly evident, but very evident 2 days later.

(continued)

TABLE XVII (continued)

Creamery Waters

Source	Sample	Tank or Well	Total Counts T.G.S.	Total Counts Gelatin	Proteolytic Counts T.G.S.	Proteolytic Counts Gelatin
AZ	61	City	1,000	1,000	270	240
	62	W	3,400	100	0	100
	63	T	130,000	30,000	60,000	11,000
BA	64	W	1,470,000	1,440,000	480,000	810,000
O	65	W	100	600	0	500
	66	T	400	300	0	100
BB	67	C.R.W.	0	20,000	0	20,000
	68	T.#1	800	700	0	400
	69	T.#2	40,000	33,400	18,000	9,000
AB	70	W	400	150	100	61
	71	T	1,900	2,500	100	700
P	72	W	300	800	0	500
	73	City	400	800	11	100
F	74	T	500	700	0	200
	75	W	36	64	6	8
	76	pre-heated	5	9	0	2
G	77	WW	21,300	32,000	1,100	13,000
	78	EW	2,600	2,500	400	1,200
	79	T	15,000	91,000	600	22,000
	80	at churn	5,100	8,800	200	1,200
BC	81	at churn	<100	<100	<100	<100
	82[1]	W	3,360,000	4,040,000	>3,000,000	3,150,000
	83[1]	W	1,310,000	1,030,000	140,000	170,000
	84[1]	W	221,000	340,000	7,000	130,000
R	85	T	24,000	77,000	7,500	12,000
	86	W	38,100	9,000	3,100	2,000
	87	W	8,100	37,000	1,600	5,000
	88	W	9,800	19,400	1,000	1,500
	89	T	17,000	120,000	13,100	29,000
	90	Churn washwater	323,000	400,000	19,000	21,000

[1] 82 is a repeat of 23
83 " " " 30
84 " " " 22

TABLE XVIII

Summary: Total count distribution for tank and well waters

Range	Total Counts Frequencies			
	Tank Waters		Well Waters	
	T.G.S. Agar	Nutrient Gelatin	T.G.S. Agar.	Nutrient Gelatin**
0- 5,000	12(34.3)*	14(40.0)	22(49.0)	17(38.6)
5,000 - 10,000	6(17.1)	5(14.3)	9(20.0)	6(13.6)
10,000 - 20,000	5(14.3)	1(2.9)	2(4.4)	8(18.2)
20,000 -100,000	6(17.1)	7(20.0)	5(11.1)	4(9.1)
100,000 - 300,000	3(8.6)	3(8.6)	3(6.7)	1(2.3)
over 300,000	3(8.6)	5(14.3)	4(8.8)	8(18.2)

* Numbers in brackets are percentages of the total number of samples of each type of water.

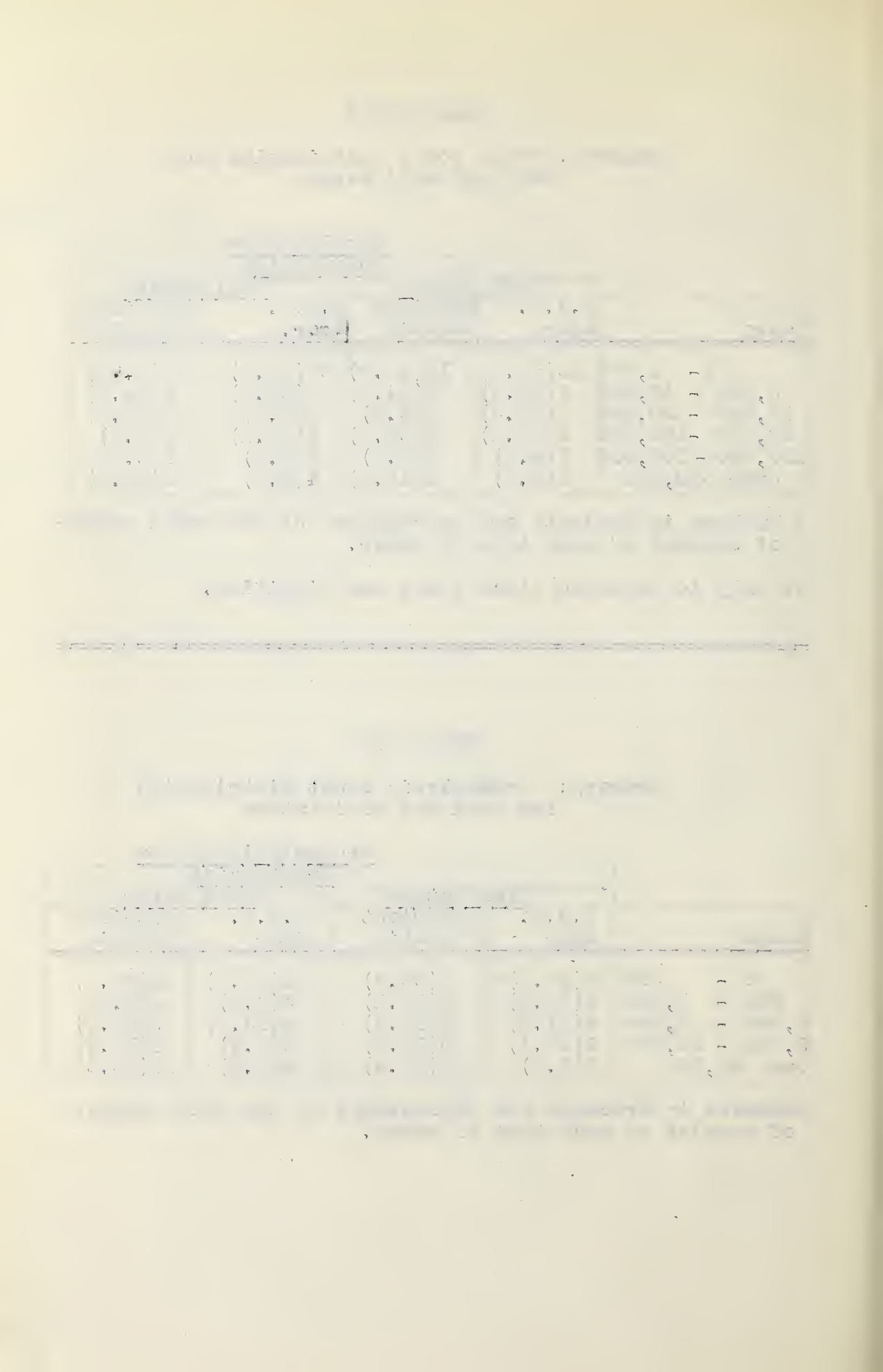
** Only 44 reported since 1 set was liquified.

TABLE XIX

Summary: Proteolytic count distribution for Tank and Well Waters

Range	Proteolytic Counts Frequencies			
	Tank Waters		Well Waters	
	T.G.S. Agar	Nutrient Gelatin	T.G.S. Agar	Nutrient Gelatin
0 - 500	17(48.5)*	8(22.8)	30(66.6)	16(35.6)
500 - 1,000	5(14.3)	6(17.1)	3(6.7)	4(8.8)
1,000 - 5,000	4(11.4)	5(14.3)	6(13.4)	9(20.0)
5,000 - 20,000	6(17.1)	5(14.3)	2(4.4)	6(13.4)
over 20,000	3(8.6)	11(31.4)	4(8.8)	10(22.2)

*Numbers in brackets are percentages of the total number of samples of each type of water.



As between tank and well waters it would seem that total counts on nutrient gelatin for tank waters were in most cases slightly higher than the same for well water. The same relation held for proteolytic counts, except that the differences on gelatin were rather more marked.

Whether the disparity in proteolytic counts as between media is due to an actually greater number of organisms which are capable of showing proteolysis as judged by gelatin liquefaction, or whether it is due to the ability of the technician to recognize proteolysis more readily on gelatin than on T.G.S. is not clear at present. However, there are grounds for believing that the former possibility may be the case since, as will be shown later, the recognition of proteolytic colonies on T. G. S. agar may be subject to wide errors.

Even though creamery waters meet public health criteria it is felt that the meeting of such requirements does not necessarily mean the absence of organisms which are important in butter deterioration.

Organisms suspected of having surface taint producing abilities, isolated from the above mentioned waters were identified as being Ach. putrefaciens or variants. From 7 well waters 11 such cultures were isolated; 11 came from 2 tank waters used as butter washwater from 2 creameries; 5 came from 2 churn washwater samples from 2 creameries; one came from the butter washwater of a creamery.

These creameries were located within a radius of approximately 150 miles and are representative of the creameries

visited. It might, therefore, be suspected that this organism had quite a wide distribution. With improved methods of isolation the ubiquity of this organism may be further substantiated.

Referring to Tables XX and XXI it is seen that well waters yielding Ach. putrefaciens or variants tended to have lower total and proteolytic counts on both T.G.S. agar and gelatin than tank waters yielding the organism. As noted previously the same relation held for all the water examined. A probable explanation is the lack of attention or suitable sanitary care accorded to tanks used as water reservoirs.

From the observation on isolations made from churn washwaters it would seem that inoculation of undesirable organisms into churns even after adequate treatment may easily contribute bacterial growth products which might be incorporated in the butter with the subsequent development of surface taint.

TABLE XX

Quantitative Data for water samples yielding *Ach. putrefaciens* or variants.

Creamery	Water Number	Source	No. of cultures isolated	Total Count		Proteolytic Count	
				T.G.S. Agar	Nutrient Gelatin	T.G.S. Agar	Nutrient Gelatin
R	87	dug well	1	8,100	37,000	1,600	5,000
	88	drilled well	3	9,800	19,400	1,000	1,500
	85	tank butter wash	10	24,000	77,000	7,500	12,000
	90	churn wash-water	3	323,000	400,000	19,000	21,000
AI	8	well	3	170,000	460,000	150,000	440,000
AJ	5	butter wash	1	312,000	420,000	205,000	120,000
AK	26	well	1	170,000	250,000	2,000	100,000
AL	-	well	1	-	-	-	-
G	77	well	1	21,300	32,000	1,100	13,000
	78	well	1	2,600	2,500	400	1,200
	79	churn wash	2	15,000	91,000	600	22,000
Z	35	tank	1	17,900	2,470,000	900	440,000

TABLE XXI

Summary of quantitative data for water samples yielding Ach. putrefaciens or variants.

	Total Count		Proteolytic Count	
	T.G.S. Agar	Nutrient Gelatin	T.G.S. Agar	Nutrient Gelatin
Well waters	2,600 - 170,000	2,500 - 460,000	400 - 150,000	1,200 - 440,000
Tank waters (including butter wash water.)	17,900 - 312,000	77,000 - 2,470,000	900 - 205,000	12,000 - 440,000
Churn wash water	15,000 - 323,000	91,000 - 400,000	600 - 7,500	12,000 - 22,000

X. STUDIES ON *Achromobactor putrefaciens*

1. Source of the culture of *Achromobacter putrefaciens* used in pure-culture work.

In the summer of 1935 Dr. J. B. Linneboe, Provincial Dairy Analyst, isolated an organism from a sample of surface taint butter made in an Alberta creamery and identified it as *Ach. putrefaciens* of Derby and Hammer. A copy of this organism was kindly supplied and (unless otherwise stated) served in all work reported on *Ach. putrefaciens*. The stock culture, carried on the usual laboratory media, was purified frequently by plating and picking in the usual way.

2. Description of *Ach. putrefaciens*

The description of the culture of *Ach. putrefaciens* mentioned above was checked, using the techniques in Manual for Pure Culture Study of Bacteria and corresponded to that given by Derby and Hammer (1931) except in one feature - acid was produced after 8 days in glucose broth containing phenol red as an indicator. In addition to Derby and Hammer's description other characteristics were recorded as below:

Growth in various media.

Growth at both room temperature and ice box temperature (10-15°C) was much better on old A.P.H.A. standard agar, veal agar or T.G.S. agar than on 1% peptone or 1% beef extract agars. Growth took place on Simmon's citrate agar, showing an alkaline reaction around each colony. No distinguishing

features of growth were noticed on Levine's eosin methylene blue agar, although there was a tendency to confluence. Growth was slow on Violet Red Bile agar (Difco), no precipitation of the bile being evident. The reddish color of the medium, immediately around each surface colony, was discharged, the "halo" becoming wider with age.

Effect of Several Dyes on the Growth of Ach. putrefaciens

1. Crystal violet in concentrations greater than 1:40,000 inhibited growth of Ach. putrefaciens in a glucose agar base. Bacto Oxgall enhanced this inhibitory effect in nutrient broth.
2. Brilliant Green inhibited growth in glucose agar in a concentration of 1:200,000. No higher dilutions were tried.
3. In tryptone broth growth was inhibited by mercurochrome in a concentration of between 1:2500 and 1:5,000. In a solid medium a concentration of 1:10,000 was found sufficient to inhibit growth of the organism.

The results with these dyes indicate that this organism has comparatively strong gram-negative characteristics. At no time in this study has it been possible to demonstrate a gram reaction other than negative for this organism.

Chromogenesis and other Photic Characters of Ach. putrefaciens

In spite of the fact that the generic name of this organism leads one to believe that no color is produced a definite color is formed. Colonies of Ach. putrefaciens on tryptone-glucose-meat extract-skimmilk agar have a brownish white color by reflected light and a transparent brownish-red color by trans-

mitted light. This holds for other solid media. There is also a marked iridescence at the edges of colonies and streaks on this medium and on others, mentioned above. Liquid media, however, were found not to bring out chromogenesis by this organism.

Lipolysis by Ach. putrefaciens

Ach. putrefaciens was found to be non-lipolytic as shown by the nile blue sulphate and natural fat methods of Long and Hammer (1937) using butterfat as the substrate.

Pleomorphism in cultures of Ach. putrefaciens.

Pleomorphic forms of Ach. putrefaciens were noticed in:

1. One percent LiCl broth.
2. Broth containing 6% NaCl.
3. Skimmilk containing 4% NaCl.
4. Various synthetic media.
5. Old broth or skimmilk cultures.

3. pH Relations

A series of McIlvaine's buffers was made up in nutrient broth, distributed in tubes and autoclaved for 20 minutes at 15 lbs. After inoculating with a loopful of a 24 hour broth culture of Achromobacter putrefaciens the broths were incubated at room temperature. The pH of a sterile control of each set was checked electrometrically at the time of inoculation and after the growth period of two days.

TABLE XXII

Growth of Ach. putrefaciens in buffered Broths.

Set No.	Initial pH	pH after 2 days Control	Inoc. tube	Growth
1.	5.14	5.08	5.08	-
2.	5.26	5.27	5.26	±
3.	5.34	5.27	5.27	±
4.	5.48	5.48	5.48	+
5.	5.76	5.67	5.67	+±
6.	6.00	5.99	5.99	++
7.	6.25	6.20	6.20	++
8.	6.50	6.51	6.51	+++

- no growth
growth, number of 's indicating strength.

From Table XXII it is seen that growth was not initiated at pH 5.14 but was at pH of 5.26. This was found to hold in a duplicate experiment. Previously (Wolochow 1938)* a rough determination of the lower limit of pH which would allow growth yielded a value of 5.6. This result was based on broth adjusted with sterile acid, the pH being taken after growth. The use of buffered broth allowed a more reliable determination. The table also shows that growth was enhanced as the pH approached neutrality - which is in agreement with Claydon and Hammer (1939).

The upper pH limit of growth of Ach. putrefaciens was determined in sterile skimmilk and in peptone water. 0.5% peptone water was made up in various buffer solutions (McIlvaine's) and sterilized by autoclaving. Skimmilk, made by reconstituting skimmilk powder on a 1:10 basis in buffer solutions, coagulated on autoclaving. Therefore it was necessary to resort to the stand-

*Acknowledgment is made for permission given by the Committee on Graduate Studies to present material preliminary to acceptance in thesis form.

ardization of pH by means of sterile 1N NaOH. The pH's of both the peptone waters and the milks were recorded with a glass electrode*. Each set was inoculated with a broth culture of Ach. putrefaciens and incubated at room temperature.

TABLE XXIII

Growth of Ach. putrefaciens at various pH's in skimmilk

Initial pH	After 2 days			After 6 days		
	pH	Growth	Odor	Growth	Odor	Odor on acidification
6.72	-	++	s.f.	++	s.f.	s.f.
7.30	7.09	++	s.f.	++	s.f.	s.f.
7.70	7.30	++	s.f.	++	s.f.	s.f.
8.25	7.62	++	s.f.	++	putrid	s.f.
8.50	7.64	++	none	++	putrid	s.f.
9.00	8.25	++	none	++	putrid	s.f.
9.50	8.54	++	none	++	putrid	s.f.
10.72	9.50	+ -	none	-	none	none

Plus and minus signs have same significance as in Table XXII.
s.f. - "Sweaty-feet" odor.

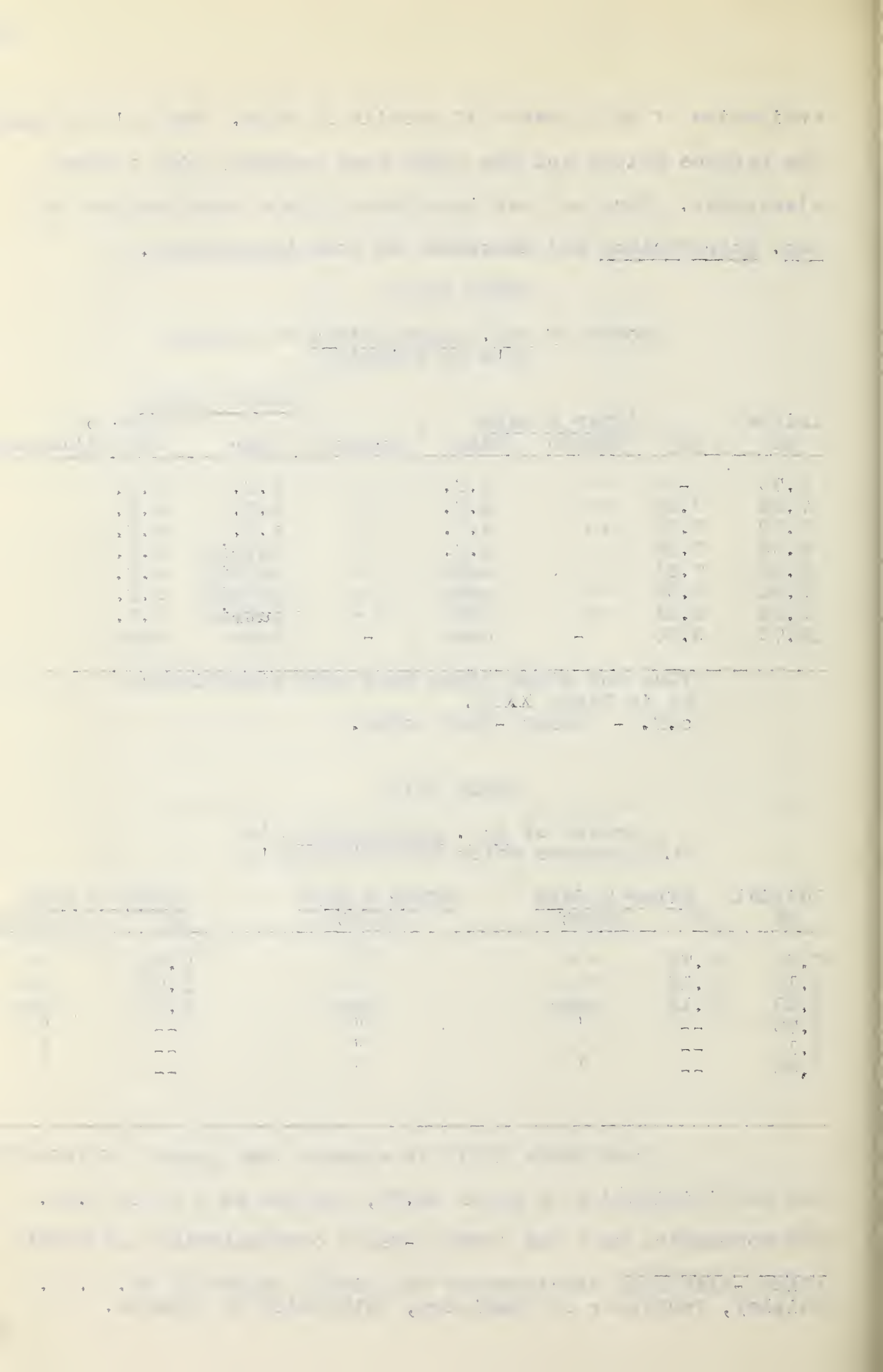
TABLE XXIV

Growth of Ach. putrefaciens in 0.5% peptone water at various pH's

Initial pH	After 2 days		After 6 days	After 9 days	
	pH	Growth	Growth	pH	Growth
7.5	7.75	++	+++	7.48	+++
8.16	8.15	++	+++	8.75	+++
8.20	8.18	none	none	8.10	none
8.72	--	"	"	--	"
9.1	--	"	"	--	"
9.32	--	"	"	--	"

From Table XXIII it appears that growth in skimmilk was not initiated at a pH of 10.72, but was at a pH of 9.50. The contention that the "sweaty-feet" odor material has acidic

*Glass-electrode electrometer was kindly loaned by Dr. J. W. Shipley, Professor of Chemistry, University of Alberta.



properties is brought out from the above data. In six days at pH 7.62 only a putrid odor was noticed, the "sweaty-feet" odor being undiscernible. Acidification released this odor and it became apparent. It is also shown that the odor of a skimmilk culture may be composed of at least two components, one of which we recognize as "sweaty-feet" and the other as typically putrid. The same facts will be shown by an aeration experiment.

In Table XXIV it is shown that growth in 0.5% peptone water was initiated at a lower pH than in milk. Growth was started at pH 8.20 but not at pH 8.72. In both the skimmilk and peptone water cultures the microscopic picture was normal.

The growth limits of Ach. putrefaciens are therefore well beyond the pH's normally encountered in butter.

An experiment was performed to show the pH - time relationship of a skimmilk culture of Ach. putrefaciens. Four ½ litre flasks, each containing 250 cc sterile skimmilk were adjusted with sterile N. NaOH at four different pH levels. Samples of each flask were removed aseptically and the pH determined with the glass electrode. Readings were taken daily for six days, at which time each flask was inoculated with a culture of Ach. putrefaciens. Inoculation was delayed in order to obtain a stable pH level and the period from 1 to 6 days acted in the nature of a control. From Chart I it will be noted that the pH of the sterile milks varied somewhat. The effect of growth of Ach. putrefaciens was to bring the pH of the milks toward a common centre, around pH 6.4 - 6.7.

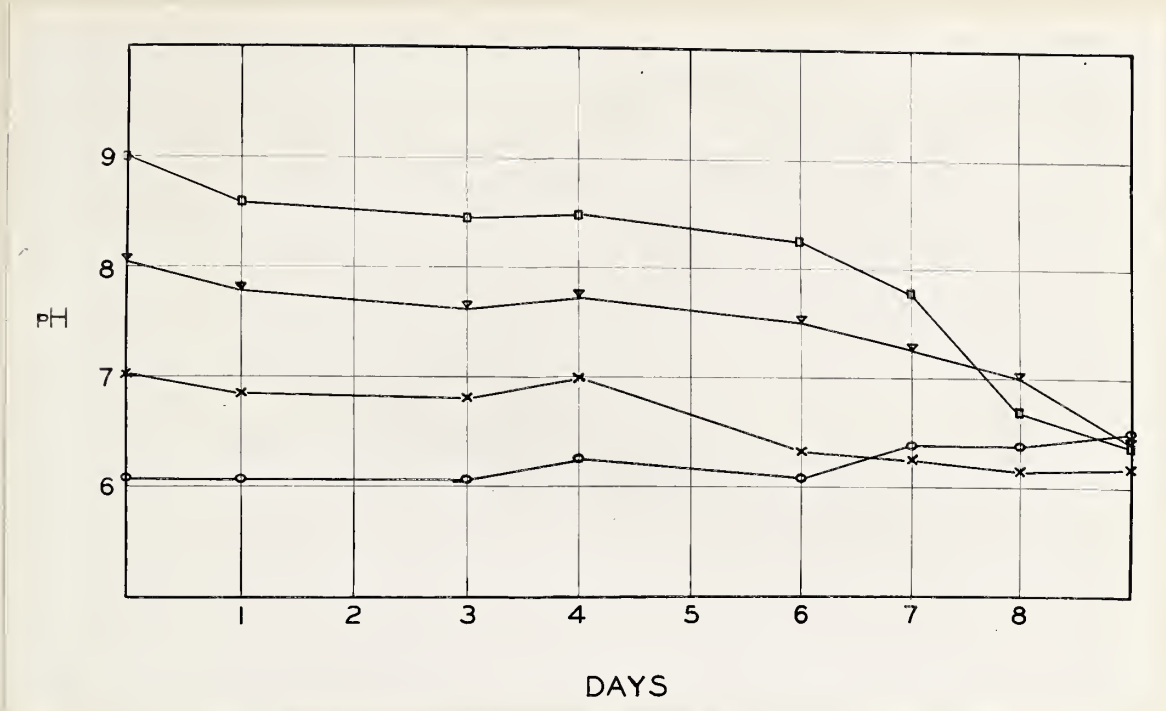


Chart I
pH: time curves of Ach. putrefaciens in milks adjusted to various pH's. (preliminary trials)

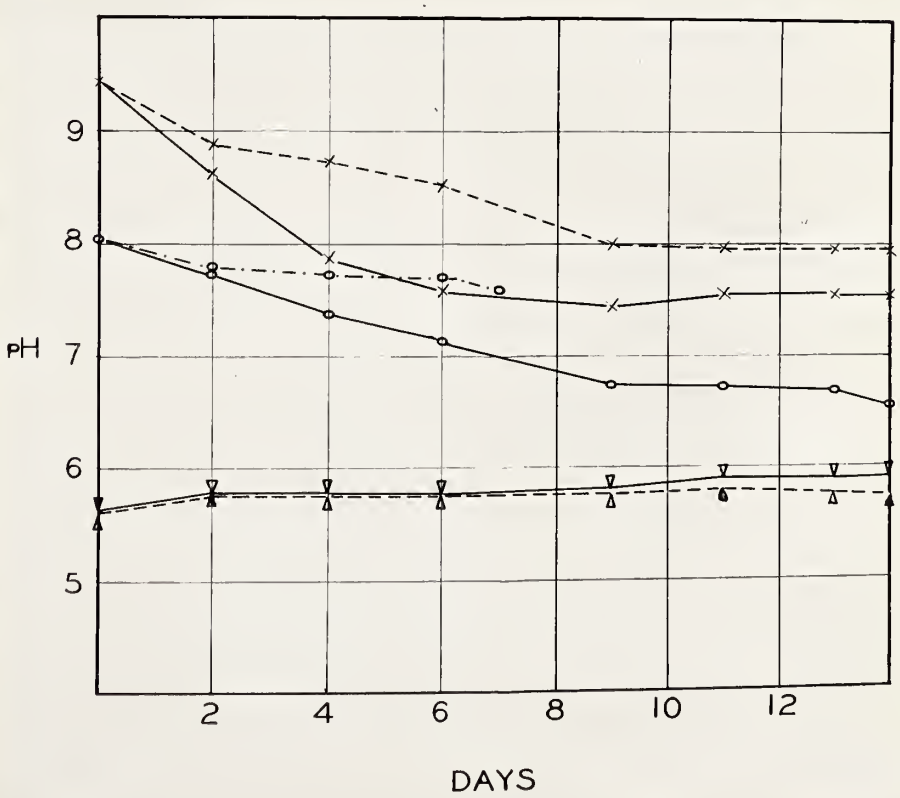


Chart II.
pH: time curves of Ach. putrefaciens in milks adjusted to various pH's.
Whole lines - inoculated samples.
Broken lines - control samples

Because the pH of the sterile milks was observed to fall with time even before inoculation it was deemed advisable to repeat the experiment, using a more adequate system of control. Three 1-litre flasks each containing 500 cc of sterile skimmilk were adjusted with sterile N. NaOH and N. H_2SO_4 to various pH's. Two hundred fifty cc were poured off aseptically into sterile flasks to serve as controls, while the remaining 250 cc were inoculated with a skimmilk culture of Ach. putrefaciens. Incubation was carried out at room temperature. pH's were determined at daily intervals with a glass electrode.

From Chart II it is seen that the rapidity of pH decrease and also the levels reached were not as marked in the second experiment as they were in the first. No explanation for this discrepancy has occurred to us. The tendency, however, is the same in both experiments. A possible explanation for this tendency is that the break-down products of the milk constituents overcame the slight buffering effect of the added NaOH or H_2SO_4 by combining with them to form weakly dissociated products and so allowing the milk to regain its original reaction.

4. E_h Relations

Ach. putrefaciens reduces litmus when grown in milk or broth containing this dye. This fact, along with several other considerations, suggested a more extensive survey of the relation of the growth of this organism to E_h.

Bright platinum-wire electrodes were stored in 95% ethyl alcohol. Just prior to aseptic introduction into the culture solution to be tested the electrode was freed of alcohol

in the flame. The unknown electrode vessel was an ordinary culture tube and was connected by a sterile KCl-agar bridge through a saturated KCl junction to a 3.5N KCl-calomel standard reference electrode. Voltages were read at frequent intervals on a K2 Leeds and Northrop potentiometer (as used in some of the pH determinations). Before assembly of the apparatus the sterile culture medium was inoculated with a broth culture of Ach. putrefaciens. Incubation was at room temperature or at 25°C in a water bath.

Chart III shows the E_h-time relations of the organism in three media. It will be noted that this organism has strong reducing properties, especially in nutrient broth, in which there is little or no poising action. In skimmilk there seems to be quite marked poising and the most negative values obtained in this medium were not as low as in the other two. This fact may have some bearing on the observation that growth appears to be more rapid in a broth medium than in litmus milk.

Claydon and Hammer (1939) report that adjustment of the E_h with various substances failed to facilitate to any marked degree the growth of the organism on agar media and did not alter the irregular results obtained on plating cultures in various dilutions. A possible explanation for these results is that the added substances were not in sufficient concentration to poise the medium at a favorable potential, since the medium, in a thin layer in contact with air, would likely have a positive E_h. More work on this point is indicated.

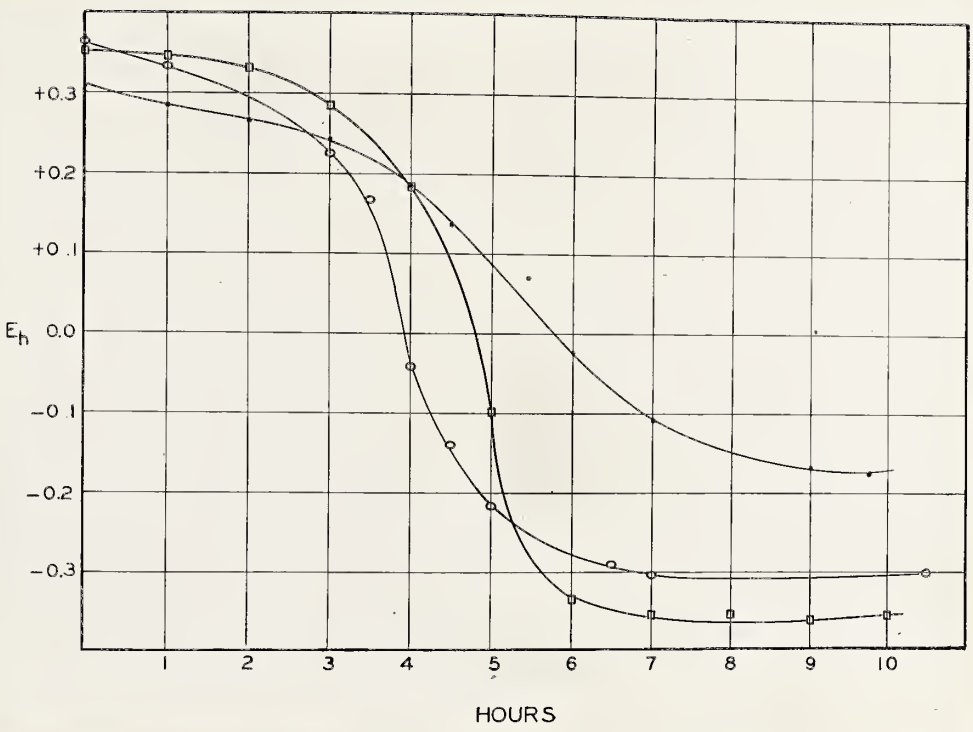


Chart III
Potential: time curves for Ach. putrefaciens

⊡—⊡ - plain nutrient broth.

⊙—⊙ - 1% glucose broth.

●—● - litmus milk.

A note on a dye reduction phenomenon.

Tubes of litmus broth were prepared to contain the following percentages of skimmilk powder: 0.8, 1.2, 1.6, 2.0, 2.4, 4.0. Following autoclaving and cooling the tubes were inoculated with a broth culture of Ach. putrefaciens and incubated at room temperature for 4 days. The litmus in all tubes was reduced at the end of this time.

Agitation caused the litmus to return to the oxidized state, presumably by the action of incorporated atmospheric oxygen. At this point it was noticed that the higher the concentration of skimmilk the more difficult it was to reoxidize the litmus. Conversely, the order of the rate of re-reduction was from the lowest to the highest skimmilk concentration. Apparently the effect of the skimmilk was to poise the medium at an E_h below that necessary for litmus reduction, which is about 0.04 volts at pH 7.0. Referring to Chart III it will be seen that in skimmilk there is a region of poising in this neighborhood. Furthermore Breed smears showed large numbers of bacteria in all tubes with no detectable differences between tubes.

These data appear to have no direct relation to the surface taint problem but they may throw some light on the mechanism involved in dye reduction in milk. The point in question is: Did the bacterial cells themselves play an active part in dye reduction (as advocated by Hobbs, 1939), or were the milk constituents responsible for dye reduction (as advocated by Jackson, 1936)? From the above data it would appear that

bacterial numbers were not significantly different from tube to tube, the variable being the concentration of the skimmilk powder. Hence it might be concluded that something in the skimmilk was contributing to dye reduction, the role of the bacteria being to lower the E_h (by removal of oxygen) to a point at which reduction could take place. This reasoning would tend to confirm the views of Jackson that bacteria play but a small part in dye reduction in milk.

5. Salt Relations

Nutrient broths, to which NaCl had been added in concentrations from 0 to 8% were distributed in tubes, sterilized and inoculated with a culture of Ach. putrefaciens. Sterile skimmilk was added in varying amounts to sterile NaCl to give similar concentrations and likewise treated.

TABLE XXV

Effect of Salt on growth of Ach. putrefaciens.

% Conc. NaCl.	Broth			Skimmilk		
	1 day	3 days		1 day	3 days	
	Turbidity	Smear	Hanging Drop	Smear	Smear	Hanging Drop
0	++	+	+	++	++	+
2	+	+	+	++	+	+
4	-	+	-	-	+	-
6	-	+	-	-	-	-
8	-	-	-	-	-	-

Turbidity and Smear - + indicates growth.
Hanging drop - + indicates motility.

From Table XXV it appears that growth was inhibited in broth containing over 6% NaCl and in skimmilk containing over 4% NaCl. Motility was inhibited by 2% NaCl in either medium.

Two samples of butter moisture each containing approximately 6.8% NaCl were sterilized in test-tubes and inoculated with the organism. A portion of one sample was diluted to 1.2% NaCl with distilled water and also treated. Growth and odor were not noticed in those tubes containing butter moisture with the higher concentration of NaCl but were in the diluted.

6. Nitrogen Metabolism

Substances released in the decomposition of milk proteins are cited as the cause of the odor of surface taint butter. Derby and Hammer assume this to be the case as did Brown (1928), although the former workers considered bacterial action in the butter as the cause, while the latter considered "extra"-butter action, with the incorporation of the decomposition products in the butter responsible. Putrid and putrefaciens themselves connote protein decomposition.

Because of the possibility that proteins or other nitrogenous material may be the source upon which Ach. putrefaciens acts to produce surface taint butter and because it was desirable to learn more of the action of Ach. putrefaciens the following experiments were performed:

1. A study of the nitrogen distribution in a skim-milk culture of Ach. putrefaciens was made.

2. A study was made of the action of Ach. putrefaciens on various nitrogenous substances both in synthetic and broth media.

1. Nitrogen Distribution Experiment.

The general method was to observe the changes in a skimmilk culture:

1. Of nitrogen not precipitated by trichloracetic acid ("NPN") - which is a measure of all nitrogen not in protein form.

2. Of "formol" nitrogen - which is measure of NH_3 and free amino group nitrogen.

3. Of NH_3 nitrogen.

Skimmilk powder was reconstituted on a 1:10 basis in N/100 NaOH, distributed in 25 cc amounts in 100 cc Erlenmeyer flasks and autoclaved at 15 lbs. for 20 minutes. After cooling each flask was inoculated with a broth culture of Ach. putrefaciens and incubated at room temperature. Sterile controls were kept. At various intervals determinations were made on a flask of culture according to the following techniques:

1. Total nitrogen was determined by pipetting 2 cc of the mixed sample into a 100 cc Kjeldahl flask in which were placed a small teaspoonful of a digestion mixture (containing K_2SO_4, $CuSO_4$, and selenium) and 2.5 cc of conc. CP H_2SO_4. The mixture was digested over a micro burner in an improvised hood until its color was a clear green. After cooling, about 60 cc of distilled water were added and when cool again, anti-bump granules. With the flask in position on the distillation stand 10 cc of conc. CP NaOH were added slowly down the side of the flask. With due care the flask was then connected to the condenser through a safety bulb. After mixing the acid and alkali the solution was heated to boiling with a micro burner and the condensed vapors collected in 10 cc of

standard N/10 H_2SO_4. About 50 cc of distillate were collected and back-titrated with standard N/10 NaOH to the methyl orange end-point.

2. Ammonia nitrogen was determined by pipetting 5 cc of the culture medium into a 100 cc Kjeldahl flask followed by about 50 cc of distilled water. Conc. NaOH was added dropwise in sufficient quantity to render the contents decidedly alkaline to litmus. Paraffin shavings were added to prevent foaming. About 30 cc. of distillate were caught in standard N/10 acid and back-titrated with standard alkali to the methyl orange end-point.

3. Nitrogen not precipitated by trichloracetic acid ("NPN") was determined on 5 cc of the filtrate obtained by adding 5 cc of culture to 5 cc of 16% trichloracetic acid, mixing and after standing for about 20 minutes filtering through a dry paper into a dry receiver. Further technique was similar to that used in the total nitrogen estimation.

4. Formol nitrogen. Five cc of culture medium, diluted with about 30 cc of distilled water were titrated to phenolphthalein end-point (pink) with alkali. Ten cc of neutral formaldehyde were then added and the mixture again titrated to phenolphthalien pink end-point. This procedure estimated both NH_3 and free amino-group nitrogen.

All estimations, after correcting for normality of acid and alkali and for dilution were expressed in terms of N/10 alkali, in 5 cc of culture medium, as a percentage of the total.

From Plate IV it will be noted that all three forms of nitrogen sustained increases with time. NPN increased most

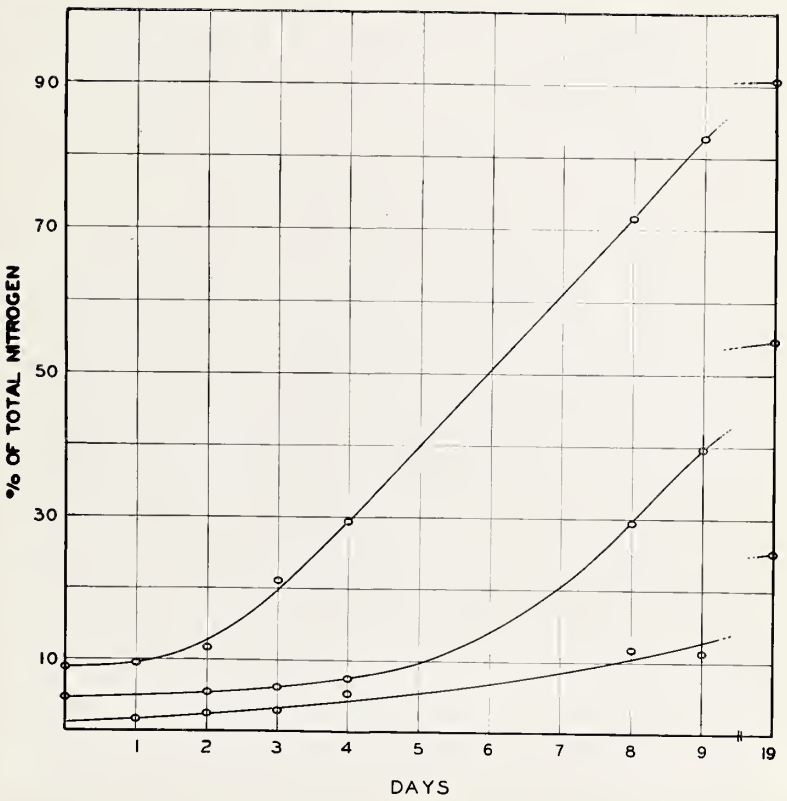


Chart IV

Nitrogen Metabolism of Ach. putrefaciens in skimmilk.

Upper curve - nitrogen not ppted. by trichloracetic acid.
Middle curve - "formol" nitrogen.
Lower curve - NH_3 nitrogen.

rapidly, formol nitrogen next and lastly NH_3 nitrogen. A logical interpretation of these curves is: The organism acted on the proteins of the milk making them soluble in trichloracetic acid, a reagent which is said to precipitate proteins only. This is then followed by a further breakdown of the complex polypeptides with a simultaneous release of free amino groups - as shown by increased formol nitrogen. Next in the series is the cleavage (presumably by reductive deaminization) of the amino groups to form NH_3.

The above is very strong evidence that this organism is indeed actively proteolytic, taking this term to mean "protein dissolving" or simplifying.

Furthermore the above reasoning offers a good explanation for the appearance of fat-solvent soluble materials in the bore of a condenser when a skimmilk culture of Ach. putrefaciens is steam distilled. As mentioned above the NH_3 is thought to come from the reductive deaminization of protein decomposition products, among which undoubtedly are free amino acids. This process would result in the formation of free fatty acids which are volatile with steam.

Of particular interest to the surface taint problem is the observation that the "sweaty-feet" odor was not noticed until the NPN began to rise steeply on the second day after inoculation. However, it should be cautioned that these may be only unrelated simultaneous occurrences, although the possibility that the odor compound arose from protein decomposition is indeed attractive.

2. Action of Ach. putrefaciens on various nitrogenous compounds.

For a discussion of the odor production by Ach. putrefaciens from various substances in broth see the section on odor production by Ach. putrefaciens.

Quite early in the work it was found that Ach. putrefaciens could grow (although but sparsely) in as simple a medium as phosphate-buffered asparagine water. The asparagine served both as a carbon and nitrogen source. It then became of interest to ascertain the availability of nitrogen from different sources and with different carbon supplies. One litre of Kisch's solution, less glucose (Levine and Schoenlein-1930) was prepared, using CP chemicals in distilled water. To 500 cc portions of this solution were added 5 g glucose and 5 g sucrose, respectively. The solutions were distributed in 50 cc lots and 1 of 9 different nitrogen sources added separately to each 50 cc. Each chemical was weighed to the nearest 0.02 g and the figures reported in Table XXVI represent the weight per 50 cc. After all the materials were in solution (or suspension) they were distributed in 10 cc lots in test tubes (plugged with cotton) and autoclaved at 15 lbs. for 20 minutes. Ach. putrefaciens was inoculated from a broth culture, 2 tubes of each set receiving two drops each of culture, 2 tubes a loopful of culture and the remaining tube serving as a sterile control. Incubation was carried out at room temperature. Growth was confirmed by microscopic examination, by smearing of the medium on agar and by subculture in litmus milk.

TABLE XXVI

Growth of Ach. putrefaciens in various synthetic media.

Carbon Supply	Time in days	Asparagine 0.3gm.	$(NH_4)_2SO_4$ 0.2 gm.	Creatine 0.5 gm.	Casein (Hammarsten) 2.0gm.	KNO_2 0.4gm.	Urea 2.0gm.	KNO_3 0.4 gm.	Na caseinate(Difco) 1.0 gm.	$NH_4H_2PO_4$ 0.2 gm.	Nothing
Glucose	8	heavy growth	growth	v.sl. growth	good growth		v. sl. growth	no growth	growth and ppt.	growth	no growth
	9	small gm. neg. rods	few sml.gm. neg. rds. and cocci	sm. gm. neg. rods and cocci	gram neg. rods normal		no visible cells	few irreg. gram neg. rods	gram neg. rods normal	small gm. negative cocci	no visible cells.
	10	Streaked on T.G.S. agar & inoculated into litmus milk. Results read 3 days later.									
	13	reduction, growth	no red.# no growth	reduction growth	reduction growth		no red. no growth	no red. no growth	reduction growth	reduction growth	no red. no growth.
	35	growth	growth	growth	growth		no growth	no growth	growth	sl. growth diacetyl smell	no growth.
Sucrose	8	very good growth	growth	growth	growth ring.	loop tube-? drop " -+	no growth	sl. growth	heavy growth	sl. growth	v. sl. growth
	9	gram neg. rods	gram neg. rods	gram neg. rods	gram neg. rods	coccoid gm. neg. rods	only few degenerative cells	gram. neg. coccoid rods	gram neg. rods	small gram neg. rods	small gram neg. rods and cocci
	10	Streaked on T.G.S. agar and inoculated into litmus milk. Results read 3 days later.									
	13	reduction growth	reduction growth	reduction growth	reduction growth	reduction growth	no. red. no growth	reduction growth	reduction growth	reduction growth	
	35	growth	growth	growth	growth	no visible growth	no growth	growth	growth	growth	growth

#red - reduction of litmus
growth - growth on plates

From Table XXVI it would seem that the nitrogen requirements of Ach. putrefaciens may be adequately met by simple compounds. This might possibly mean that this organism is, in truth, heterotrophic and therefore should be placed in the Family Pseudomonadaceae. (Bergey-1939). More work, involving the serial transfer in a given medium, is indicated as a means of settling this point, since it was noted that those tubes receiving the large inocula showed slightly better growth.

7. Sulfur Metabolism

Derby and Hammer make no mention of H_2S production by Ach. putrefaciens. In the present work it was found that, while growth of the organism for 6 days in Pb acetate agar Difco failed to show H_2S production, peptone water cultures containing either cysteine or cystine (100 mg.%)emitted H_2S, the presence of which was confirmed by a Pb acetate paper test and by smell. A similar culture of methionine yielded a negative H_2S test.

For a study of the H_2S production from skimmilk powder and cystine in both aqueous and broth media the following solutions were prepared:

(1) (a) Water to which varying amounts of skimmilk powder were added. (Table XXVII).

(b) Broth to which varying amounts of skimmilk powder were added. (Table XXVII).

(2) (a) Broth to which varying amounts of skimmilk powder were added, in the presence of 0.12% added cystine. (Table XXVIII).

(b) Water to which 10% skimmilk powder was added in the presence of from 0.00012 to 0.012% added cystine. (Table XXIX).

TABLE XXVII

"Sweaty-feet" and H_2S production in broth and skimmilk powder and in diluted skimmilk.

Skimmilk powder %	In Broth: 1 day Growth	7 days S.F.	19 days S.F.	19 days H_2S
0.0	partially reduced	-	-	-
0.8	reduced	-	-	+
1.2	"	-	-	+
1.6	"	-	±	+
2.0	"	+	±	+
2.4	"	-	±	+
4.0	"	±	+	-
		+	+	-

	In Water: 1 day Growth	12 days S. F.	H_2S
0.0	not reduced	-	-
0.6	" "	-	-
1.0	" "	-	-
1.4	" "	-	-
1.8	" "	-	-
2.1	" "	-	-
3.8	" "	±	-
10.0	reduced	+	-

[illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]

TABLE XXVIII

"Sweaty-feet" and H_2S production
in broth + skimmilk powder and cystine

Skimmilk powder conc.	Cystine conc. %	1 day		7 days		16 days	
		Growth	H_2S	S.F.	H_2S	S.F.	H_2S
0	0	+	-	-	-	-	-
	0.012	+	++	-	+	-	+++
0.1	0	+	-	-	-	-	-
	0.012	+	++	-	++	-	+++
	0.0312	+	-	-	-	-	-
1.0	0	+	-	-	-	-	+
	0.012	+	+	-	++	-	+++
	0.0012	+	-	-	+	-	+
2.0	0	+	-	-	-	-	++
	0.012	+	+	-	+	-	+++
	0.0024	+	-	-	+	-	+
5.0	0	+	-	-	-	-	++
	0.012	+	-	±	-	-	+++
	0.006	+	-	-	+	-	+
10.0	0	+	-	+	-	+	-
	0.012	+	-	+	-	+	±

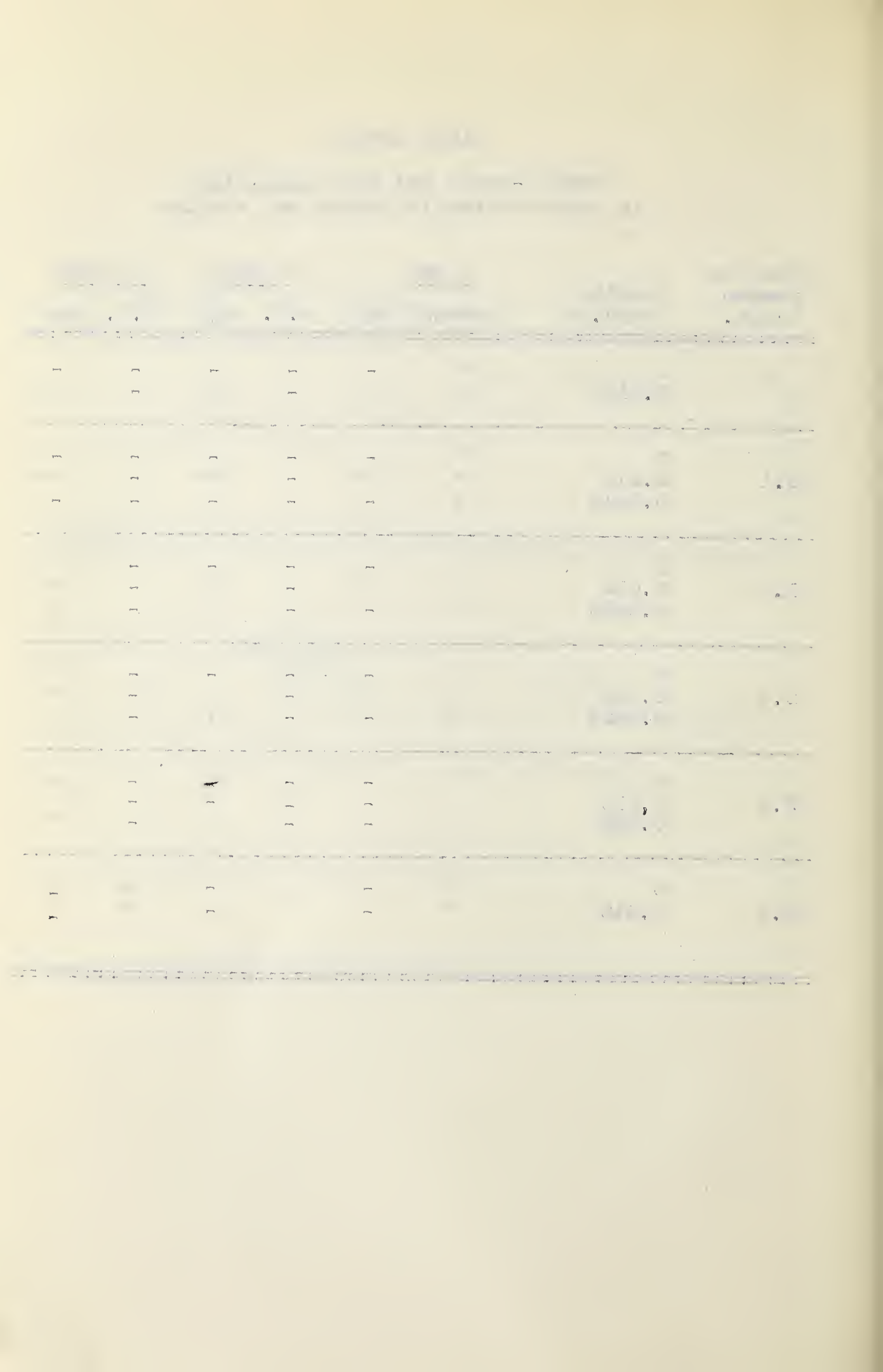


TABLE XXIX

"Sweaty-feet" and H_2S production in skimmilk + cystine.

Skimmilk powder %	Cystine %	1 day Growth	2 days Growth	7 days S.F.	7 days H_2S
10	0.00	partially reduced	partially reduced	+++	-
10	0.00312	" "	" "	+++	-
10	0.0012	" "	" "	+++	-
10	0.0024	" "	reduced	+++	-
10	0.006	" "	"	+++	-
10	0.012	" "	"	+++	-*

* Slight H_2S produced after 15 days, but rest were negative.

TABLE XXX

H_2S production as affected by skimmilk and cystine.

In Broth

Skimmilk powder %.	cystine - %. 0	0.00012	0.0012	0.0024	0.006	0.012
0.0	-					+++
0.1	-	-				+++
1.0	+		+			++
2.0	++			+		++
5.0	++				+	+
10.0	-					+

In Water

0.6	-
1.0	-
1.4	-
1.8	-
2.2	-
3.8	-
10.0	- - - - - +

+ - H_2S produced
- - H_2S not produced.

[illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

[illegible]

[illegible]

[illegible]	[illegible]	[illegible]

[illegible]

(c) Broth containing varying amounts of skimmilk powder in varying amounts of added cystine. (Table XXVIII).

Fifty cc lots of each member of a series were prepared, each member containing litmus to serve as a growth indicator. After distribution in 10 cc lots in test-tubes the solutions were autoclaved at 15 lbs. for 15-20 minutes. Inoculation was from broth cultures and incubation was carried out at room temperature. The Pb acetate paper method was used for testing for H_2S production. A sterile strip of the test paper was inserted aseptically between the neck of the test tube and the cotton plug so that it projected into the space above the medium. A test was considered positive if the paper showed blackening about the edges and the intensity of production was noted by the distance the black color extended up the paper.

From Tables XXVII to XXX it is seen that:

1. H_2S was not produced in a water solution of 0-10% skimmilk powder.
2. H_2S was not produced in nutrient broth.
3. H_2S was produced in nutrient broth when 1.0% to 5.0% skimmilk powder was added. In dilutions outside this range H_2S production was not found.
4. The addition of 0.012% cystine to nutrient broth containing from 0 to 10% skimmilk powder enabled the organism to produce H_2S, although its production was delayed in those broths containing 5% to 10% skimmilk powder.

5. H_2S production was observed in 10% skimmilk powder in water with 0.012% added cystine. Even in this case H_2S production was delayed considerably. H_2S production was not observed if the concentration of cystine was 0.066% or less.

Other sources from which H_2S was produced by Ach. putrefaciens.

1. Skimmilk containing from 27.9 ppm. to 0.003 ppm. $FeSO_4$.

2. Skimmilk powder reconstituted 1:10 in 0.005N $Na_2S_2O_3$.

3. "Rennet" whey neutralized to pH 7.0 with sterile NaOH after autoclaving.

4. An autoclaved solution of egg albumen prepared by suspending flake egg albumen in water.

Discussion on the Sulfur Metabolism of Ach. putrefaciens.

The sulfur metabolism of Ach. putrefaciens was studied primarily with the object of discovering the probable source of the odoriferous substance responsible for surface taint butter. Referring to Tables XXVII to XXIX it is seen that there is a tendency for the production of the "sweaty-feet" material and the production of H_2S not to occur simultaneously. From a metabolic point of view this might possibly mean that the substance responsible for odor production has something to do with the sulfur metabolism of the organism, either as an intermediate to H_2S production, or as a diversion in its "normal" metabolism. It is difficult to say at present whether H_2S production or "sweaty-feet" production is the "normal" channel of metabolism.

We cannot advance a satisfactory explanation for all the observations made on the sulfur metabolism of this organism. It would seem that the organism is able to split off H_2S from cystine and this splitting is inhibited by some constituent in

skimmilk powder. The nature of the inhibition may be: 1.- the skimmilk removes the cystine (e.g. by adsorption) so that it is unavailable to the organism; or 2.- the skimmilk supplies a more readily-available hydrogen acceptor. To complicate the picture there appears to be a source of H_2S in the skimmilk itself; Plimmer and Lowndes (1937) report 0.032% cystine in cow's milk. A nice balance between the H_2S source and the H_2S-suppressing source must be obtained before H_2S can be liberated from the skimmilk without the addition of cystine. The effect of $FeSO_4$ might have been to catalyse the liberation of cystine from milk proteins, making it available for the organism as a source of H_2S. This also appears to be the case with $Na_2S_2O_3$. The supposition that the source material of the H_2S is in the albumen fraction of the milk proteins is somewhat confirmed by the fact that H_2S was obtained by the action of the organism on "rennet" whey and on egg albumen, both of which contain combined cystine. In the case of the "rennet" whey, which was sterilized in acid solution (pH 6.2), it is believed that part-hydrolysis occurred with the liberation of cystine.

7. Aeration of a Skimmilk Culture of Ach. putrefaciens

The primary object of these experiments was to find a possible method of obtaining the material responsible for "sweaty-feet" odor in as simple a solution as possible. Observations of marked importance to the whole problem of surface taint butter made on these experiments will be presented below.

Basically, the idea was to pass an air current through a skimmilk culture of Ach. putrefaciens and attempt to catch volatile growth products in some simple medium.

Three attempts were made but only one will be described. Alongside the Ach. putrefaciens set-up a parallel experiment was run with B. subtilis (from a stock laboratory culture) as the inoculum.

Plate I is a diagrammatic sketch of the equipment used. All parts of the apparatus were sterilized in the autoclave, except those which obviously did not require it. Air from the pressure line was passed through two wash-tubes containing fairly concentrated NaOH into 200 cc of inoculated litmus milk contained in a 500 cc Kjeldahl flask. The purpose of the litre flask between the culture flask and the absorption tubes was to prevent any milk from reaching the absorbing reagent, since the milk frothed if the air were passed too rapidly through the system. The absorbing medium, dilute NaOH, was contained in two absorbing tubes connected in series. NaOH was used since it has been shown (Dunkley-1940) that the "sweaty-feet" material has acidic properties.

During aeration at room temperature the following observations were made:

1. Litmus in the Ach. putrefaciens culture remained reduced once growth had taken place for the period observed.
2. NH_3 (by Nessler test) was produced by both organisms.
3. Surface tension of the Ach. putrefaciens culture was markedly increased after one day's growth, as evidenced by lessened foaming of the medium.
4. No H_2S was detected by the lead acetate paper method in the air coming from either culture.

5. No indole could be detected by the Ehrlich-Böhme test in the air coming from either culture.

6. "Sweaty-feet" odor could be detected before, but not after passage through NaOH of air from the Ach. putrefaciens culture.

7. The air coming from the Ach. putrefaciens culture after passage through the NaOH solution had an odor typical of putrefaction.

8. Air coming from the B. subtilis culture had no "sweaty-feet" odor both before and after passage through NaOH.

While, as pointed out above, the "sweaty-feet" and putrefactive odors were distinguishable with ease by those associated with this study, they could not be so distinguished by several practical butter men.

After 9 days the air-stream was discontinued, the purity of cultures checked by gram stains, and the following procedures carried out:

1. The contents of the culture flasks were submitted to steam distillation and the distillates, caught in $Ba(OH)_2$, were evaporated to dryness on the water bath.

2. The contents of the absorption tubes were evaporated to dryness on the water bath.

3. Acidification of the crystalline residues yielded the following:

	residue of distillate	residue of absorption tubes
Ach. putrefaciens.	"sweaty-feet" odor	Sl. "sweaty-feet" odor
B. subtilis	sharp, pungent, fatty-acid odor, but no "sweaty-feet" odor.	no "sweaty-feet" odor

The results gained in the other two aeration experiments, in which only Ach. putrefaciens was used, followed along similar lines.

From these experiments it is concluded that:

1. The metabolism of Ach. putrefaciens is such as to produce the"sweaty-feet" odor along with typical putrefactive odors.
2. The putrefactive odors may easily be confused with the "sweaty-feet" odor.
3. B. subtilis, a common putrefactive organism, does not produce the "sweaty-feet" odor but does produce fatty-acid-like odors. This is a step in the proof of our belief that the "sweaty-feet" odor production may be unique to Ach. putrefaciens.
4. The aeration method is not suitable for the mass production of the "sweaty-feet" material.

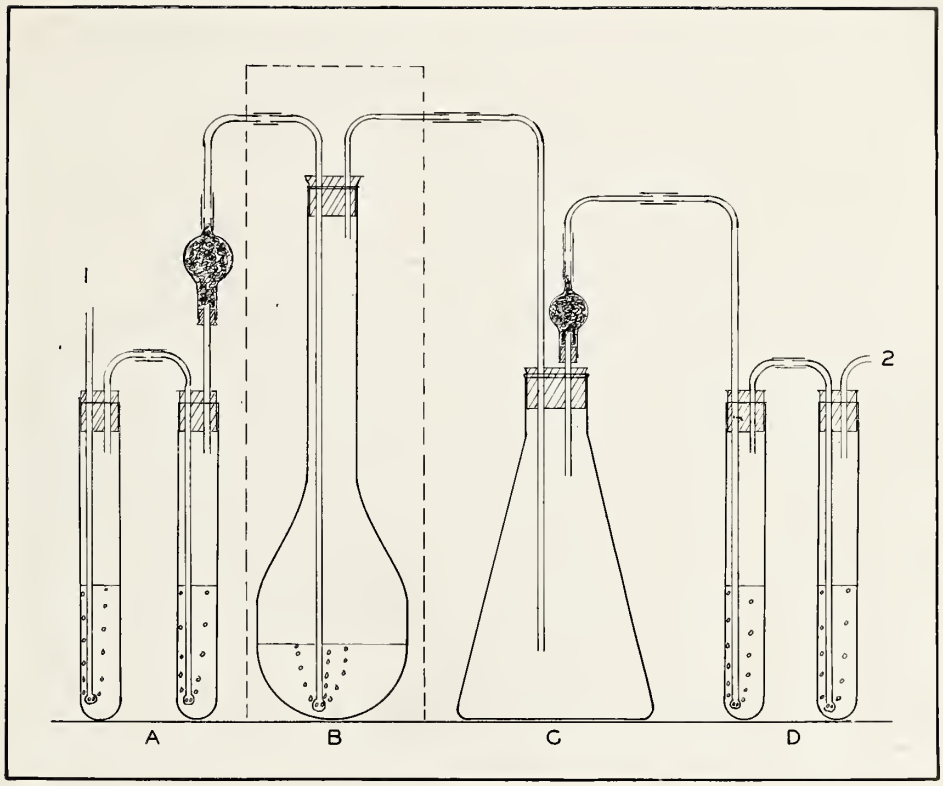


PLATE I
Aeration Apparatus

A - wash train containing NaOH
B - Culture flask
C - "safety" flask
D - absorption train containing NaOH
1 - Air inlet
2 - Air exit

XI. RELATION OF VARIOUS FACTORS TO ODOR PRODUCTION BY ACH. PUTREFACIENS.

A. Milk, Skimmilk and Cream.

1. The "sweaty-feet" odor.

As previously mentioned the odor production by Ach. putrefaciens in litmus milk was used in the search for the organism. It will be recalled that the odor was intensified when allowed to dry on the fingers to the point of stickiness. The intensification of the odor was not evident immediately after washing the hands. Suspecting that the skin secretions facilitated the release of the odor, attempts were made at similar intensification in petri dishes. Young skimmilk cultures of the organism were poured into petri dishes on the bottom of which had been spread butter, butter-oil or lecithin. The reaction of the culture was also adjusted in a set of petri dishes. All treatments failed to intensify the odor.

Preliminary work on the isolation of the "sweaty-feet" odor material indicated that it was volatile with steam. Dunkley (1940) was able to obtain a crude substance as a barium salt, giving negative tests for P, S, N and the halogens, from which the "sweaty-feet" odor could be obtained by acidification with a strong acid. Unsuccessful attempts were made to identify the surface taint and "sweaty-feet" odors with many known chemical compounds. One trial in particular deserves mention. Since Ach. putrefaciens is a proteolytic organism there is a possibility that the odor produced in skimmilk might be indole. Sterile skimmilks containing 0, 2 and 20 mg %

indole were inoculated with Ach. putrefaciens and incubated at room temperature. By smelling the tubes and by spreading the culture on the fingers it was clearly seen that the "sweaty-feet" odor and indole were not identical, for both could be distinguished with ease. Indole alone was noticed in broth cultures containing 2 and 20 mg % indole.

2. pH.

In the study of the relation of Ach. putrefaciens growth to pH it was found that the odor produced in skimmilk could be divided into at least two distinct components, depending on the pH. At a pH of about 7.6 or higher a putrid odor was noticed while the "sweaty-feet" odor, characteristic of growth at lower pH's, was not forthcoming. However, the "sweaty-feet" odor material was produced but was presumably bound up as an odorless salt, for acidification of the culture released it.

3. E_h.

It was early noticed that the "sweaty-feet" odor could not be discerned in a litmus milk culture unless growth had proceeded beyond the litmus-reduction stage.

Various methods were used to change the E_h of samples of reduced litmus milk cultures of Ach. putrefaciens:

1. Heating at 82°C for 10 minutes followed by cooling caused the litmus to be reoxidized to the blue stage. At the same time there was a loss of the "sweaty-feet" odor, which did not reappear on exposure to air.

2. H_2O_2 added to a reduced culture reoxidized the litmus and

caused the disappearance of the "sweaty-feet" odor which only slowly reappeared on exposure to air.

3. Quinhydrone addition elicited the same phenomena as with H_2O_2.

4. Litmus was slowly reduced in a litmus milk culture of Ach. putrefaciens incubated at room temperature in an atmosphere of hydrogen gas. The "sweaty-feet" odor was not noticed immediately the culture was removed from the hydrogen atmosphere but soon appeared when the medium was equilibrated with air by shaking.

When any of the above samples from which the odor had been lost was spread on the fingers the "sweaty-feet" odor could be discerned.

Another experiment showed that H_2O_2, added to $1\frac{1}{2}$ days-old litmus milk culture which was too young to give off an odor, tended to cause the "sweaty-feet" odor to become evident. The results, however, were not clear-cut.

An explanation for the above phenomena may involve the following considerations: The "sweaty-feet" odor material is produced and held in milk solution in a non-odorous reduced form. Exposure to air oxidizes this material to an odorous stage. Furthermore, it would seem that too drastic oxidation will reversibly change the odorous compound to a non-odorous compound, which when it becomes equilibrated with air, again becomes odorous.

4. Numbers.

It was found that a Breed count of at least 50,000,000 per cc of litmus milk was required before reduction of litmus took place. As previously mentioned the "sweaty-feet" odor was not noticed unless growth had proceeded beyond the litmus-reduction stage.

5. Heat Treatment.

Brown (1928) mentioned that the "disagreeable aroma" was not noticed in raw cream or raw-cream-butters. Parker (1939) threw suspicion on high temperature pasteurization as a contributing factor in the appearance of surface taint in butter. In preliminary communications (Wolochow, 1938 and 1939) the relation of heat treatment of milk, cream and skimmilk to "sweaty-feet" production by Ach. putrefaciens was reported.

Aseptically-drawn milks were obtained from two healthy cows (Holstein and Jersey) on each of three occasions. Samples of the whole milk, skimmilk and cream (gravity separated) were aseptically dispensed in sterile test tubes. Before inoculation with a broth culture of Ach. putrefaciens various heat treatments were given to the samples:

1. No treatment - raw.
2. Heated to 145°F for 10 minutes.
3. Heated to 145°F for 30 minutes.
4. Heated to 160°F for 10 minutes.
5. Heated to 180°F for 10 minutes.
6. Heated to 248°F for 10 minutes.

After incubation at room temperature for various periods the samples were tested organoleptically. Only those samples which had been heated to 145°F for 30 minutes or at higher temperatures yielded typical "sweaty-feet" odors. The raw samples and those heated to 145°F for 10 minutes emitted various odors none of which suggested the "sweaty-feet" odor.

6. Chemicals.

"Sweaty-feet" odor production in skimmilk by Ach. putrefaciens was inhibited by:

1. 0.00034N hydroquinone
2. 0.002N $CuSO_4$.
3. 0.01N $FeSO_4$.

but not by:

1. 0.005N KCN
2. 0.002% diphenylamine
3. 0.01N Na_2SO_3.

B. Butter.

1. Buttermaking technique - general.

Sweet cream (approximately 32% butterfat) was pasteurized at 180°F for 10 minutes in an improvised assembly, pictured in Plate I. The cream, contained in a covered stainless steel pail, was heated in a tub of steam-heated water. A motor actuating a stainless steel stirrer assured adequate agitation during heating and cooling. The temperature of the cream was indicated by a long-stem mercury thermometer. Cooling, first with tap water and then with ice, was accomplished in the same set-up. The pail and cover were autoclaved prior to use while the stirrer was steamed.

By means of a sterile dipper the cooled cream was transferred in 600 cc lots to 8 two-quart Perfect Seal fruit jars, in which the cream was churned. After the required treatment the jars of cream were placed at 10-15°C for approximately 24 hours.

Churning was accomplished by strapping the jars to the cross-arms of a circular-motion shaker, pictured in Plate II. This system frequently proved ineffective in "breaking" the butter, necessitating manual shaking of each jar. Following churning to wheat grain size the jars of butter in the buttermilk were placed at 10-15°C for 1-2 hours to temper the butter granules. In all cases, unless otherwise specified, 2 portions of 600 cc each of sterile tap water at the temperature of the buttermilk were used to wash the butter.

In the first part of the work the mechanical worker (sterilized by autoclaving) pictured in Plate III was used. Due to difficulty in maintaining a sterile worker for each sample the "concussion" method of working was resorted to. By means of sterile tongue depressors the gathered lump of butter was vigorously slapped on a wet, sterile, pine board until working and moisture inclusion were judged adequate. The worked butter was then formed into a pat and placed in a sterile 1 lb. ointment jar. With the cover removed the jars of butter were placed at 10-15°C and left over night, at which time each sample was removed from its container to a sterile wet board, trimmed with a thread to present a fresh surface and then returned to a fresh sterile jar, this time covered with a paper-lined lid. Incubation, unless otherwise stated, was at 10-15°C.

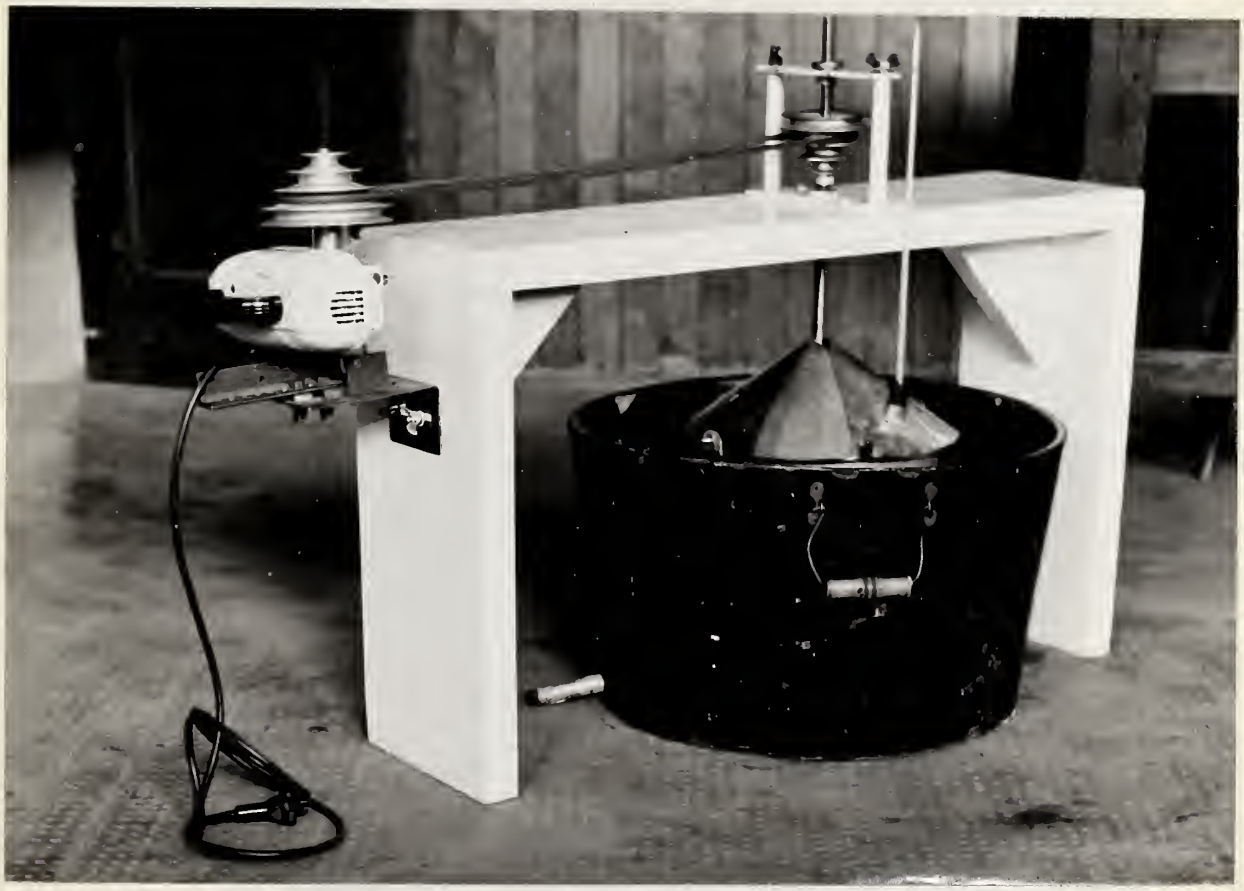


PLATE I

Pasteurizer Assembly.

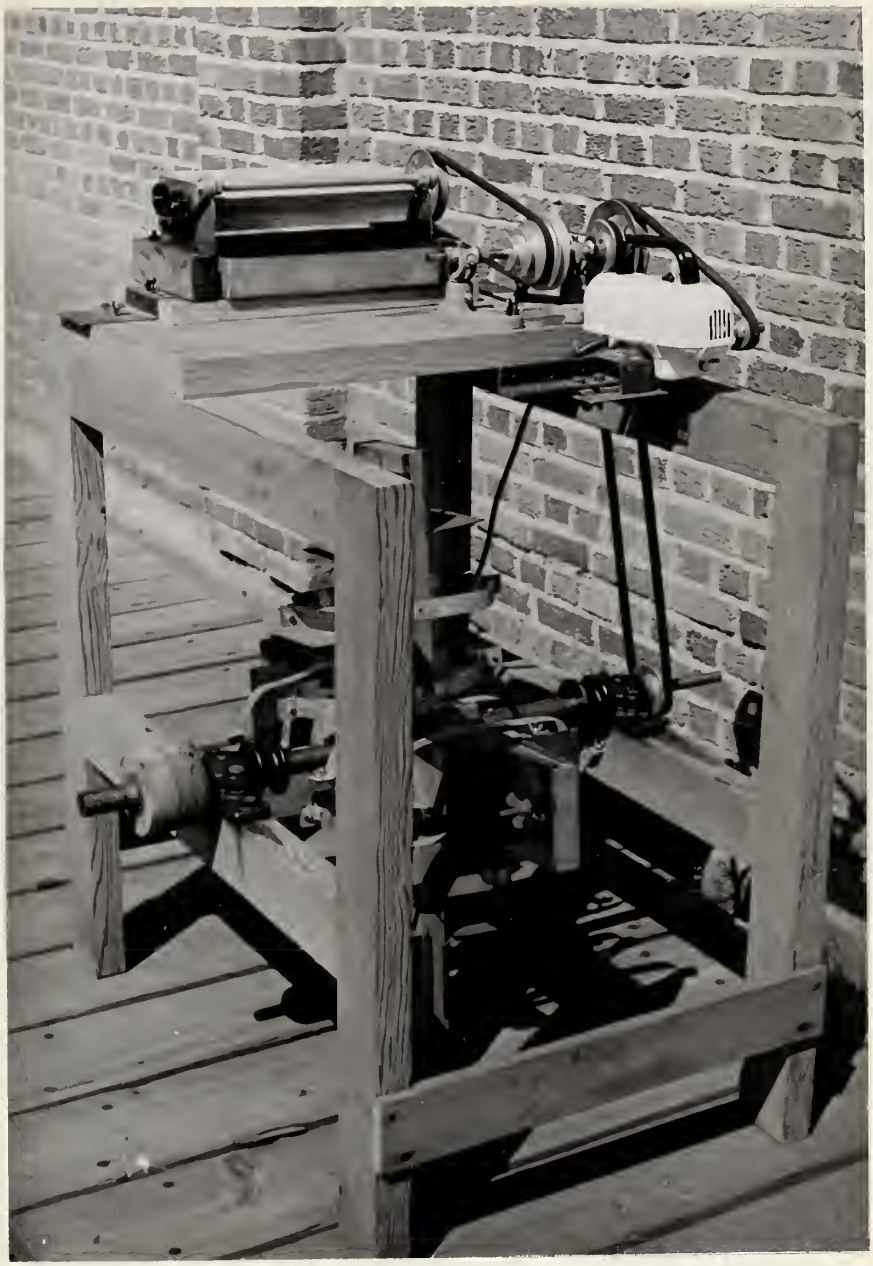


PLATE II

Experimental Churn.

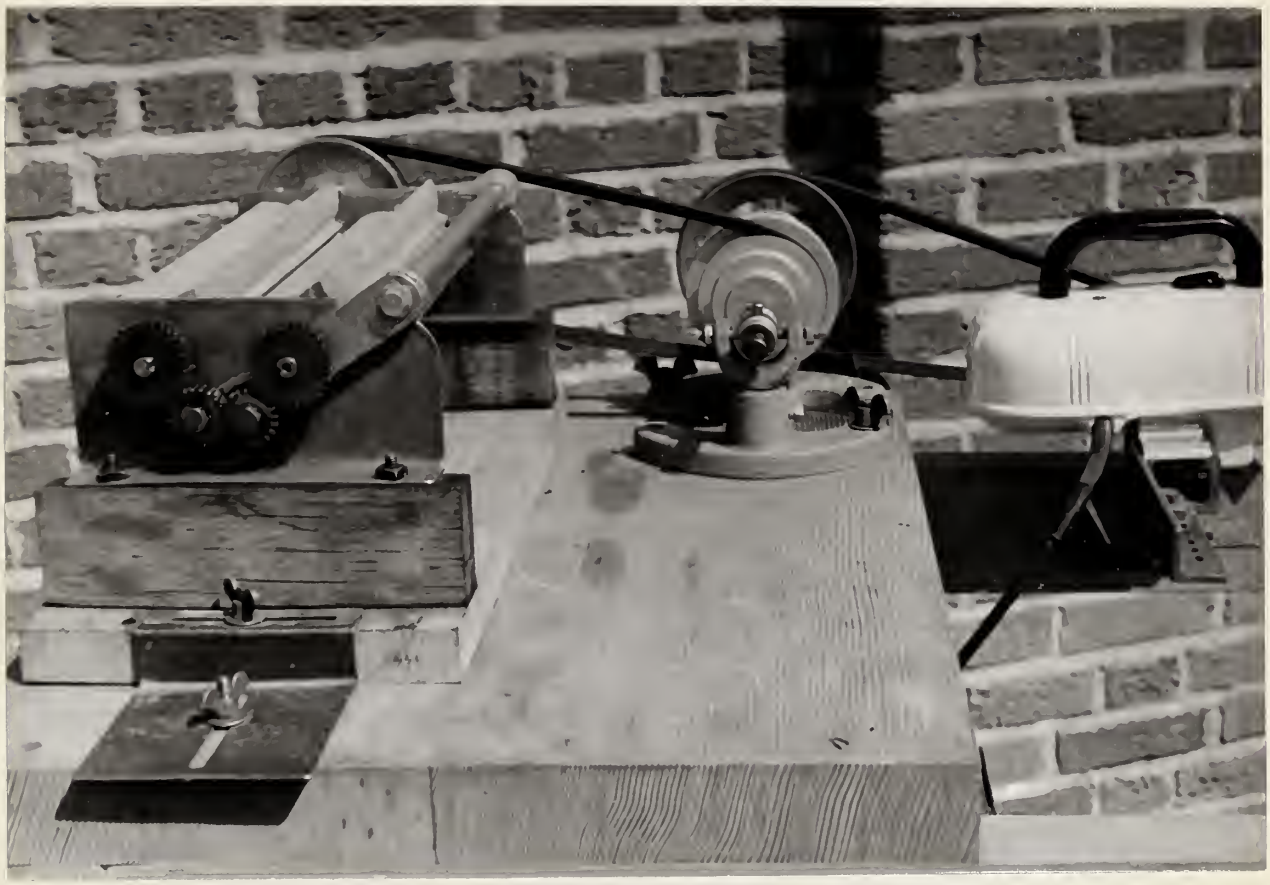


PLATE III

Details of Worker Assembly

The butters were graded by one or all of the persons associated with this work.

From the history of the growth of bacteria in butter and from the discussion which followed it will be realized that this method of making butter on an experimental scale may be open to question as to texture and moisture inclusion. Again we caution too rigid an interpretation of some of the results obtained by experimental churnings. However, at the outset of this work it was realized that it would be desirable to duplicate as closely as possible the texture, moisture inclusion and surface conditions of commercial butter. Most of the butters made by the above technique were judged by competent observers to be of fair texture but inclined to show some free moisture, especially noticeable on salted butters.

2. Pasteurization.

One gallon of cream approaching aseptically-drawn quality was treated as follows: One half was pasteurized at 180°F for 10 minutes, the other half being kept raw. Details of subsequent treatment are best obtained in Table XXXI, which also includes the grading summary. Bacteriological work showed that Ach. putrefaciens was present in all those butters which had been inoculated.

TABLE XXXI

Grading of butters made from raw and pasteurized cream inoculated with Ach. putrefaciens

Days after churning	Raw cream butter				Pasteurized cream butter			
	Control	% Nacl.			Control	% Nacl.		
	0	0	1.5	2.5	0	0	1.5	2.5
1						S.T.		
2	clean	rancid	clean	clean	clean	S.T.	S.T.	v. sl. S.T.
3	"	"	"	"	"	S.T.	S.T.	S.T.
4	sl.rancid	"	sl.rancid	v.sl. rancid	"	S.T.	S.T.	S.T.
6	rancid	"	"	"	"	S.T.	S.T.	S.T.
8	"	"	"	rancid	"	S.T.	S.T.	S.T.
11	"	"	"	"	"	sl.S.T.	S.T.	S.T.
12	Fresh surfaces exposed with a thread.							
13	clean?	sl.rancid	clean	clean	clean	sl. tallowy	S.T.	S.T.
14	rancid	rancid	rancid	"?	"	"	sl.S.T.	sl.S.T.
16	"	"	"	" ?	sl. rancid	"	sl. rancid	sl. S.T.

It will be noted that in the case of raw cream butter rancidity rather than surface taint was encountered. This experiment confirms our suspicion that heat treatment of cream was in some way responsible for the sudden occurrence of surface taint butter in 1919, shortly after the general introduction of high-temperature pasteurization in the Province of Alberta. It appears to correlate with the results obtained on the odor production by Ach. putrefaciens in raw milk, skimmilk and cream, in which a "cowy" rather than "sweaty-feet" odor was noticed.

3. Pasteurization of Cream after growth of Ach. putrefaciens.

One gallon of pasteurized cream was treated as indicated in the scheme below:

1st day	2nd day		3rd day
pasteurized cream	uninoculated	1 - churned	
		2 - repasteurized	------ churned
	inoculated*	3 - repasteurized	----churned. All 0% NaCl.
		4 - repasteurized	----churned. All 0% NaCl.
		5 - repasteurized	----churned. All 0% NaCl.
		6 - churned	0%NaCl
		7 - churned	1.5%NaCl
		8 - churned	2.5%NaCl

*3,4,&5 were in bulk at this stage, and inoculated with 3cc's of a 24 hr. broth culture, while 6,7,&8 each received 1 cc of the culture.

Between the first and second and between the second and third days the creams were kept at 10-15°C. The buttermilks from the repasteurized cream-butters were plated and streaked on T.G.S. agar and were found to be almost sterile.

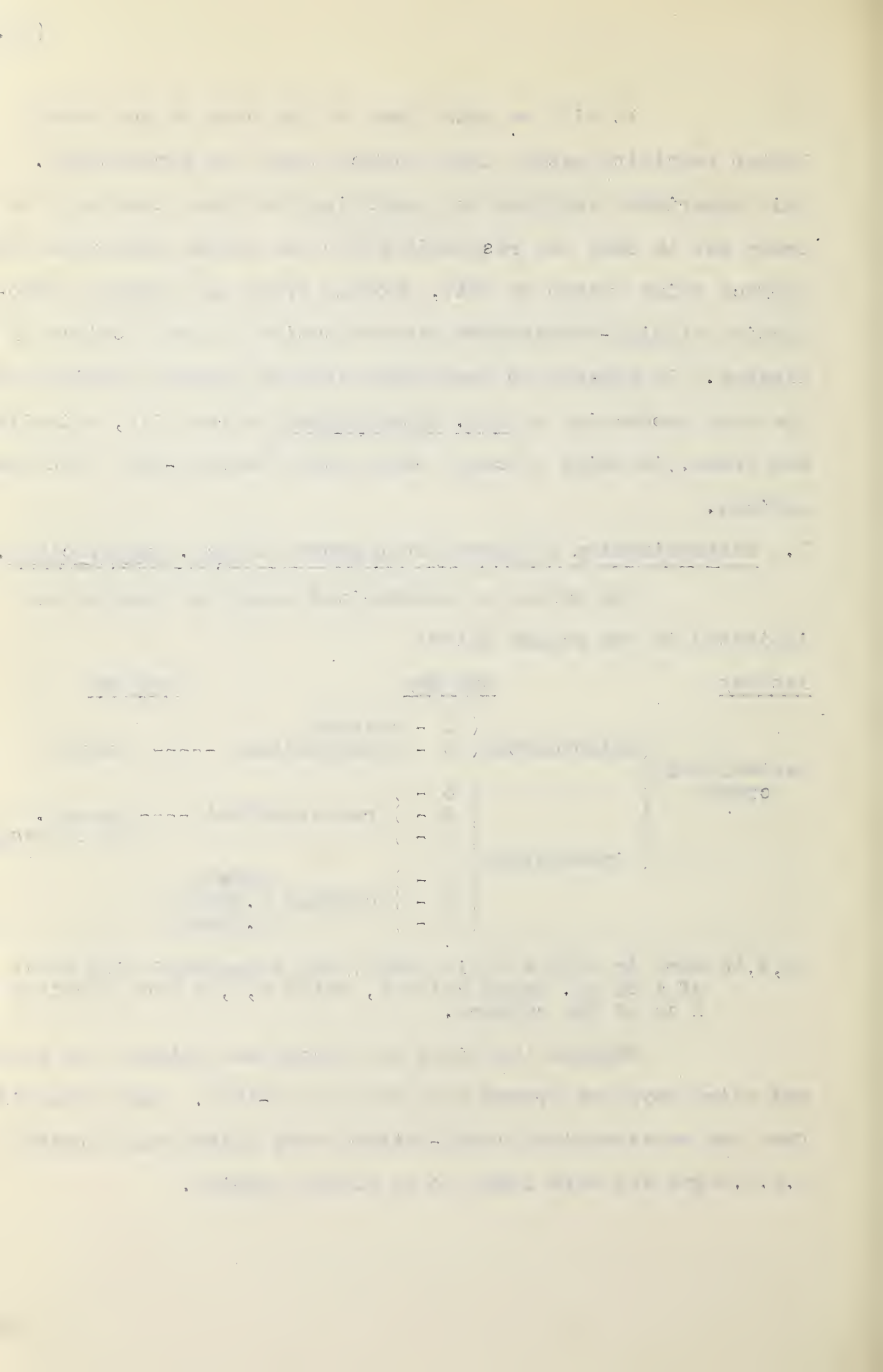


TABLE XXXII

Grading of butters made from pasteurized inoculated cream and butters made from cream repasteurized after the growth of Ach. putrefaciens

Days after Churning	Pasteurized-cream Butter				Repasteurized-cream Butter			
	Control		% NaCl.		Control		% NaCl	
	(1)* 0	(6) 0	(7) 1.5	(8) 2.5	(2) 0	(3) 0	(4) 0	(5) 0
28-30 hrs.	Clean	S.T.	v.sl. S.T.	clean	clean	clean	clean	clean
2	"	"	sl.S.T.	sl.S.T.	"	"	"	"
3	"	"	" "	" "	"	"	"	"
4	"	"	S.T.	S.T.	"	"	"	"
5	"	"	"	"	"	"	"	"
6	"	"	"	"	sl. unclean	rancid ?	rancid ?	rancid

*Numbers in brackets refer to numbers in scheme above.

From Table XXXII it is seen that pasteurization of the cream after growth of Ach. putrefaciens had taken place resulted in butters which were free from surface taint, while those samples in which the organism had grown but which were not repasteurized yielded typical surface taint butters.

From this experiment it is concluded:

1. That the presence of Ach. putrefaciens in the cream coming to the creamery is not likely to cause surface taint in the butter made from such cream providing it is adequately pasteurized and protected from subsequent recontamination.

2. That heat-treatment of cream in some way destroys or inactivates the odor-material produced by Ach. putrefaciens so that surface taint is not noticed on butter made from such heat-treated cream. In opposition to this statement it might be argued that the odor-material was not produced in the cream prior to repasteurization (or to churning), but that subsequent growth of the organism in or on the unrepasteurized-cream butters, took place. If growth did take place in the butter the mechanism involved will only be explained when more adequate observations are made.

4. Anti-oxidants.

Cream was pasteurized at 180°F for 10 minutes in lots of 600 cc. An Avenex #3 extract prepared as directed by the manufacturers was added to each lot of cream just prior to pasteurization.

1. No Avenex.
2. No avenex.
3. One-half the directed amount.
4. The directed amount.
5. Twice the directed amount.
6. Three times the directed amount.
7. Approximately 0.1% hydroquinone, no Avenex.) Added immediately before churning.
8. Approximately 0.05% hydroquinone, no Avenex.)

Jars 2 to 8 were inoculated with 1 cc of a broth culture of Ach. putrefaciens and allowed to incubate as usual.

TABLE XXXIII

Grading of butters made from pasteurized cream containing anti-oxidants.

Days after churning	Sample No. 1	2	3	4	5	6	7	8
2	clean	S.T.	S.T.	S.T.	S.T.	sl.S.T.	clean	sl.S.T.
3	"	"	"	"	"	"	"	clean
5	"	"	"	"	"	" +	sweet-ish & rancid	sweet-ish & rancid
6	"	"	"	"	"	" +		

From Table XXXIII it appears that the production of surface taint by Ach. putrefaciens was somewhat inhibited by the presence of added Avenex extract or by hydroquinone. Avenex, an oat-flour product, is used in various processes because of its anti-oxidant properties. Hydroquinone, because of the lability of its alcoholic hydrogens, acts as an anti-oxidant and apparently is oxidized in preference to the surface taint material. The oxidation of the added hydroquinone was visually confirmed by the presence of a brown-colored layer (presumably quinone) at the surface of the butter. This layer deepened as the butter aged, indicating a slow penetration of atmospheric oxygen. The delay in surface taint production was ascribed to the anti-oxidant properties of the Avenex and the hydroquinone.

Bacteriological analyses of representatives of these butters are contained in the section "Quantitative and Qualitative Changes of Bacteria in Experimental Butter". It was found that the numbers of bacteria increased with time and

also that Ach. putrefaciens was isolated with relative ease from the butters made from inoculated cream.

5. Salt.

Pasteurized-, inoculated-cream butters were salted at the rate of 0%, 1.5% and 2.5% on the expected butter yield. Kohman analyses for three butters showed that the actual salt concentration was below the calculated.

TABLE XXXIV

Grading of Inoculated butters containing salt.

Days after churning.	% salt added 0 Trial I	0 Trial II	1.5 Trial I	1.5 Trial II	2.5 Trial I	2.5 Trial II
28 hrs.	S.T.	S.T.	very sl.S.T.	sl. S.T.	clean	clean
2	"	"	sl.S.T.	S.T.	v.sl. S.T.	v.sl. S.T.
3	"	"	"	"	sl.S.T.	S.T.
4	"	"	S.T.	"	S.T.	"
5	"	"	"	"	"	"
6	"	"	"	"	"	"
11		sl.S.T.		"		"
13		sl.tallowy		"		"
16		do.		sl. rancid		sl.S.T.
Moisture	14.1%		12.9%		12.8%	
Salt	0%		0.82%		1.60%	
Curd	1.50%		0.63%		0.81%	

Salt apparently delays the production of surface taint, although the concentrations used did not eliminate it. These results are in agreement with those of Claydon and Hammer (1939), Turgasen (1939), Hood and White (1928), and our own observations on commercial surface taint butters.

6. Wash-water Contamination.

Measured amounts of either a broth or a skimmilk culture of Ach. putrefaciens were added to the second portion of water used to wash pasteurized-cream butters. The butter granules were allowed to stand in the contaminated wash-water for approximately 5 minutes prior to draining and working.

From grading results in Table XXXV it would appear that contaminated wash-water may cause surface taint to develop. This phenomenon, also noticed by Claydon and Hammer (1939), is at variance with practical observations, such as the illustration cited in the discussion of the growth of bacteria in butter. Again no explanation is forthcoming.

While Claydon and Hammer experienced difficulty in isolating Ach. putrefaciens from surface taint butter made by inoculating the wash-water, we were able, by the poured plate technique, to isolate the organism in almost pure culture from those butters which exhibited the defect.

7. Butter-working Surface Contamination.

A sample of butter churned from pasteurized cream was worked on a board which just previously had served for the working of a sample of butter churned from cream inoculated the previous day with Ach. putrefaciens. Both samples of butter

TABLE XXXV

Grading of butters washed with contaminated water.

No. of organisms introduced into wash water#	Broth Culture						Skimmilk Culture	
	0	78,000	780,000	7,800,000	78,000,000	780,000,000	11,000,000	110,000,000
days after churning	grade							
1½	clean	clean	clean	clean	S.T.?	S.T. ?	S.T.?	S.T.?
2	"	"	"	sl.S.T.	S.T.	S.T.	S.T.	S.T.
3	"	"	"	" "	"	"	"	"
4	"	"	sl.S.T.?	S.T.	S.T.strong	S.T.strong	S.T.strong	S.T. strong
6	"	"	clean	clean	S.T.	S.T.	sl.S.T.	S.T.

#Plate counts on T.G.S. agar.

developed surface taint in 48 hours and contuned to show the defect for at least six days.

Two possibilities suggest themselves, namely, 1. the organism was picked up from the contaminated buttermilk left by the inoculated sample, grew in the butter, and produced the defect, or, 2. odoriferous growth products contained in the buttermilk were left on the board and were picked up by the next sample of butter.

Extensive bacteriological work on these samples, set forth in Charts V and VI, revealed that growth of organisms (as measured by the plate count) took place and that Ach. putrefaciens constituted the major part of the total bacterial flora.

8. Numbers.

Bacterial plate counts were made soon after experimental butters had been judged to have surface taint. By picking colonies from the plates into litmus milk the approximate proportion of Ach. putrefaciens was estimated. Assuming, 1) 12% moisture in these butters, 2) that all the bacteria were in the moisture, 3) that the plate count represents individual cells rather than bacterial clumps and 4) that all viable cells grew on the agar plate, calculations showed that Ach. putrefaciens counts from 48,000 to 23,000,000 per cc of butter moisture might be expected. Previously it was mentioned that a Breed count of over 50,000,000 per cc of skimmilk culture was necessary before the "sweaty-feet" odor was noticed. This apparent disparity in numbers may be due to a number of factors, among which are:

1. Active bacterial growth may not be necessary for the production of surface taint.

2. Ach. putrefaciens may be present in butter moisture in comparatively large clumps - a factor which would falsely minimize the extent of their presence. On the other hand inclusion of viable cells in the butterfat would tend to raise the apparent numbers in the moisture.

3. As Hammer and Claydon (1939) contend, Ach. putrefaciens does not readily initiate growth on media.

C. Whey

1. Acid Whey

Whey was obtained from milk by precipitation of casein by H_2SO_4 and filtering. Following neutralization to pH 6.8 - 7.0 and heat sterilization, the whey was inoculated with Ach. putrefaciens and incubated at room temperature. In 9 days only a slight "sweaty-feet" odor was present when the culture was tested by spreading on the fingers.

2. Rennet Whey.

Whey from the Gouda cheese-making process was filtered and treated as indicated in Table XXXVI, from which it is also seen that in rennet whey only a slight "sweaty-feet" odor was inconsistently produced.

TABLE XXXVI

Odor production by Ach. putrefaciens in rennet whey.

Treatment	pH	Time of incubation in days. 3	12
Autoclaved without preliminary neutralization	6.2	v.sl. S.F.	v.sl. S.F.
Neutralized to pH 7.4, filtered and autoclaved	6.8	no S.F.	no S.F.
Neutralized after autoclaving	7.0	no S.F.	no S.F. H_2S +++

From the seeming lack of odor production in whey it might be inferred that the material being acted upon by the organism to produce the "sweaty-feet" odor resided in that fraction precipitated by either acid or rennet.

D. Butter Moisture.

"Sweaty-feet" odor was produced in 3 days by Ach. putrefaciens in a sample of sterile diluted butter moisture containing 1.2% NaCl.

E. Nutrient Solutions Containing Various Substances. General Method of Study.

Measured amounts of amino acids, fatty acids and various other substances, were placed in solution in sterile 0.5% Bacto peptone water, the reaction adjusted when necessary to pH 6.8-7.2, the solutions distributed in sterile test tubes in replicate, autoclaved at 15 lbs. pressure for 10-12 minutes and inoculated with young broth cultures of Ach. putrefaciens.

TABLE XXXVII

Odor production by Ach. putrefaciens in the presence of various substances.

"Sweaty-feet" odor

Not produced	Questionably produced
1. Proteins and allied substances	
Edestin (Difco) 0.25%	
Casein (Hammarsten) 2%	
Na caeinate (Difco) 1%	Na caseinate 2%
Egg albumin (Pfansteil) saturated solution	Peptonized milk 0.7%
Lactalbumin solution - raw and denatured	
Lactoglobulin solution - raw and denatured	
2. Amino Acids - all in a concentration of 100 mg %.	
B-alanine	
alanine	
arginine	
cysteine	
cystine	
glutamic acid	glutamic acid
glutamic acid (0.35%)	
Isoleucine	
leucine	
lysine	
methionine	
norleucine	
threonine	
tryptophane	
tyrosine	
valine	
3. Fatty Acids - all in a concentration of 0.05 cc %.	
butyric acid	
caproic "	
caprylic "	
capric "	
oleic "	oleic acid
4. Other Compounds and Materials.	
Glutamine (100 mg %)	
Glucosamine (0.35%)	
Lecithin (0.25% & 0.01% in nutrient broth.	
Peptone water 0.5%	Peptone water 0.5%
Peptone water 5.0%	
Nutrient broth	
Tryptone broth	

Proteins and derivatives other than amino acids were examined in water solution for sources of the "sweaty-feet" odor. At various intervals tests were made for odor production by smelling the culture spread on the fingers.

From Table XXXVII it is seen that of the substances tested not one gave a definite clue as to the possibility that it may serve as the source material from which the "sweaty-feet" odor is elaborated by the action of Ach. putrefaciens in milk. At various times and to various persons concerned with this work the odor of cultures containing some of the substances may have suggested "sweaty-feet". At no time was it possible to demonstrate its presence in any way approaching that produced in skimmilk cultures. Upon this point there was complete agreement.

XII. QUANTITATIVE AND QUALITATIVE CHANGES OF BACTERIA IN EXPERIMENTAL BUTTERS

A study of the quantitative and qualitative bacterial changes in experimental butters was made in an effort to: 1) determine the extent of growth in such butters; 2) determine the relative ease of isolation of Ach. putrefaciens from such butters; 3) test the adequacy of the "proteolytic" plate count as used in other studies, insofar as it reflects the possible presence of Ach. putrefaciens.

Butters were made as previously described and plated at intervals on T.G.S. agar in duplicate per dilution. Incubation of the plates was either at 25°C or 10-15°C for 4 days. From 25 to 30 random surface and subsurface colonies, from the plates of each sample of butter at each interval, were picked into tubes of litmus milk which were then incubated at room temperature for approximately one week. Those tubes showing reduction of the litmus and a cleared top layer were considered to contain Ach. putrefaciens cultures. Occasionally such a tube was smelled and a gram stain made of the contents; invariably the characteristic odor of "sweaty-feet" was obtained and the smear showed gram negative rods.

The logarithm of the total and of the "proteolytic" counts were plotted separately against time. The proteolytic count as a percentage of the total count was plotted against time, as was also the percentage proportion of the number of Ach. putrefaciens cultures picked to the total number picked.

SERIES I.

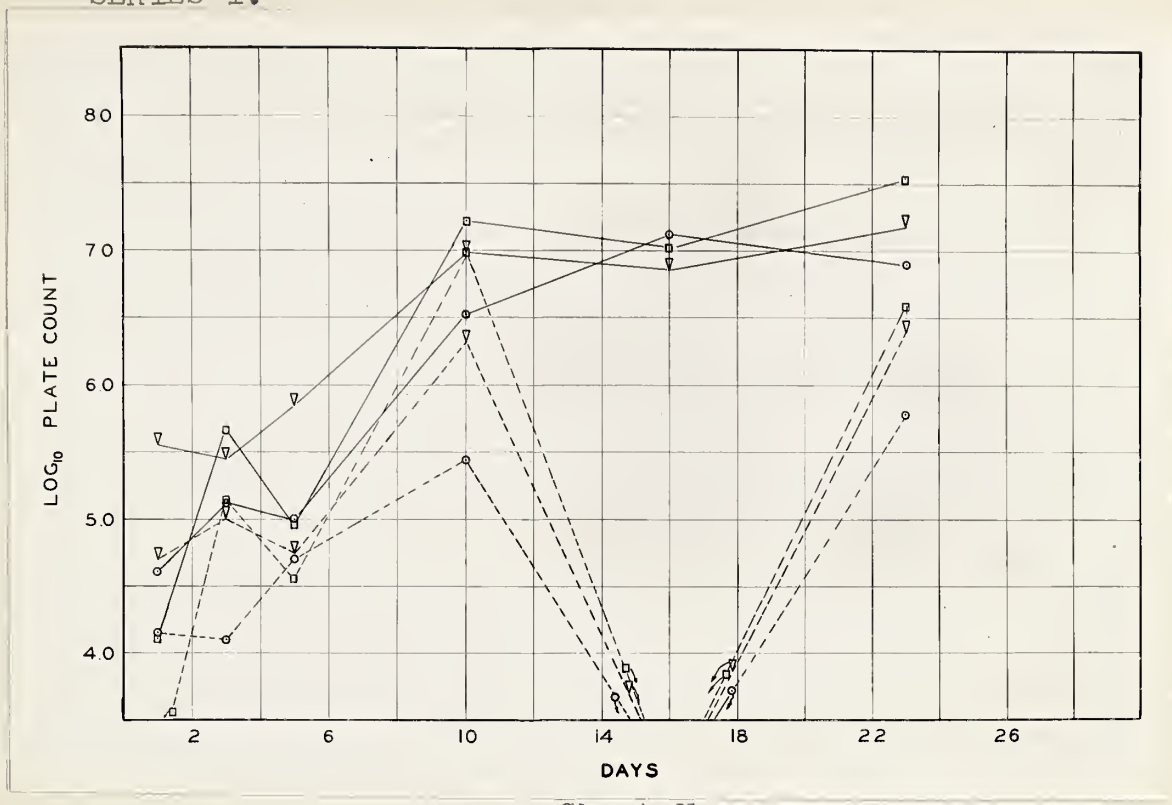


Chart V.

Showing the variations of total and proteolytic counts in three samples of butter.

Solid lines - Log total counts on T.G.S. agar incubated at 25°C.
Broken lines - Log proteolytic counts.
⊙ - Inoculated butter containing normal amount Avonex.
▽ - Inoculated butter
⊡ - "Sterile" control, worked on a contaminated board.

An inspection of Charts V and VI reveals that all samples showed essentially the same trend, namely, 1) variable increases in total and proteolytic counts, 2) a higher proportion of Ach. putrefaciens than would be indicated by the proteolytic counts.

Periodic examinations in uninoculated control butters of both Series I and II showed that, by the method used, Ach. putrefaciens was absent while the total counts increased in somewhat the same order as in the inoculated samples.

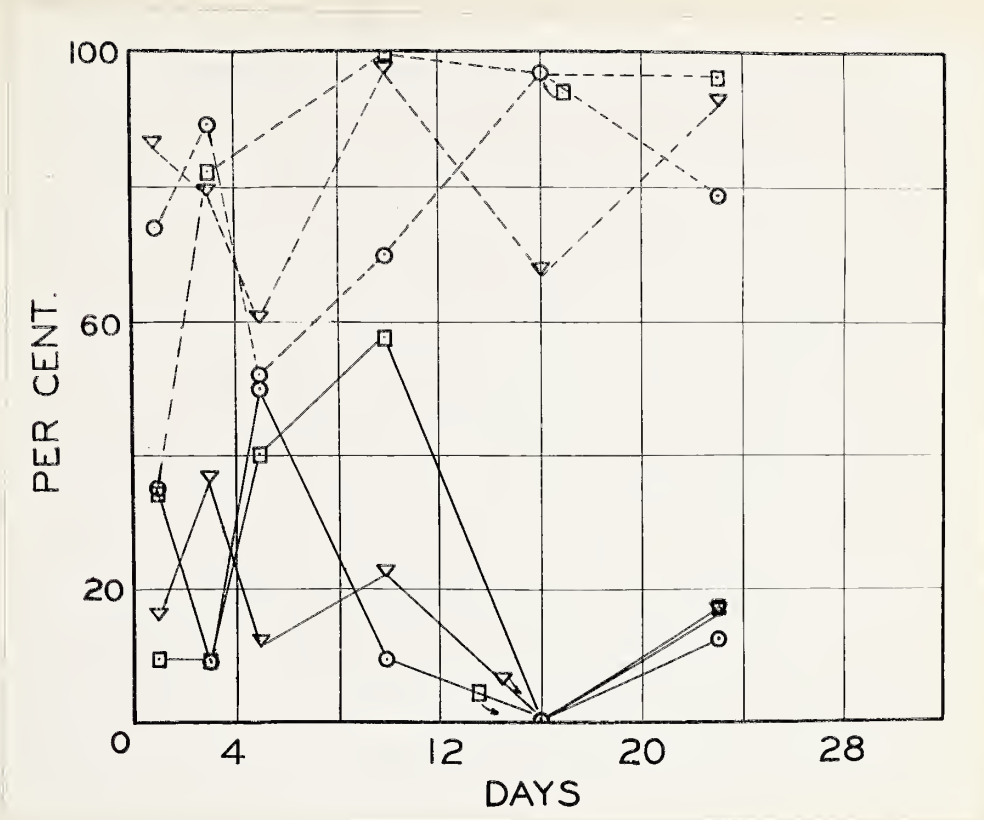


Chart VI

Showing the variations in proteolytic counts and in Ach. putrefaciens in three butters.

Solid lines - Proteolytic counts as percentages of total counts.
Broken lines - Percentage Ach. putrefaciens picked.
⊙ - Inoculated butter containing normal amount Avenex.
▽ - Inoculated butter.
⊡ - "Sterile" control, worked on a contaminated board.

SERIES II.

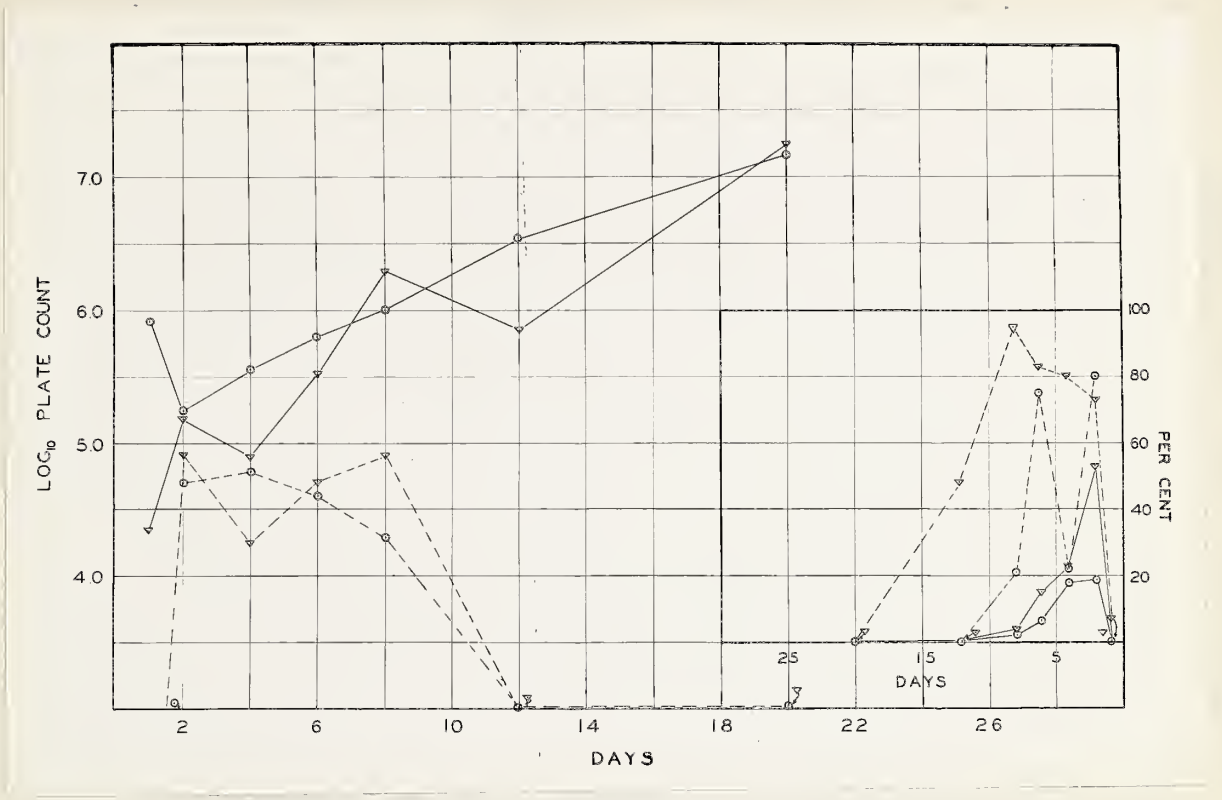


Chart VII

Main Chart. Showing variations of total and proteolytic counts in two samples of butter.
Solid lines - Log total counts on T.G.S. agar incubated at 10-15°C.
Broken lines - Log proteolytic counts.

Inset Chart. Showing the variations in proteolytic counts and Ach. putrefaciens in two butters.
Solid lines - Proteolytic counts as percentages of total counts.
Broken lines - Percentage Ach. putrefaciens picked.

Both Main and Inset Charts.
▽ - Inoculated butter.
⊙ - Inoculated butter to which 0.1% hydroquinone had been added just prior to churning.

From Chart VII it may be seen that essentially the same conditions prevailed in these two butter samples as were found in those of Series I.

From these data it is seen that:

1. There is a tendency for bacterial numbers, as shown by the plate count method, to increase in:

   (a) butters made from cream inoculated with Ach. putrefaciens,

   (b) butter which had been contaminated with Ach. putrefaciens during the working process.

2. Neither Avenex nor hydroquinone had an appreciable effect on bacterial numbers as shown by the plate count method.

3. The "proteolytic" count was not entirely satisfactory in reflecting the presence of Ach. putrefaciens which is a proteolytic organism.

4. Ach. putrefaciens was readily isolated from the butters examined.

In evaluation of the above observations it is necessary to consider:

1. That the physical and chemical conditions in the butters examined may not have been representative of commercial butters and therefore conclusions based on these observations may not apply to commercial butters.

2. That the fairly large inocula used in these butters may be entirely out of proportion to the inocula just necessary to produce the defect in commercial butters.

It is believed that the unrepresentative nature of the proteolytic count as compared to Ach. putrefaciens isolations is due to the inability and variability of the operator to spot colonies showing proteolytic clearing of the casein, especially when the colonies are close together. This fact is further aggravated by subsurface colonies, since it was noticed that

bacteria in such colonies of Ach. putrefaciens do not show up as being proteolytic.

XIII. RELATION OF Pseudomonas fluorescens TO SURFACE TAINT.

Several investigators have ascribed butter defects suggestive of surface taint to the action of Ps. fluorescens, which was apparently called Bacillus florescens liquefaciens in the early literature. Rice (1937) of Australia and Shutt (1929) of Canada claimed that this organism was responsible for "rabbito" and "surface flavor" in butter. Derby and Hammer (1931) on the other hand, found that while an objectionable condition which may have resembled surface taint for 2 or 3 days was produced by this organism in butter, rancidity was soon evident and it persisted for long periods.

Organisms with the general property of producing a greenish diffusable pigment in agar or litmus milk culture were isolated in comparatively large numbers from both butters and waters. Most of such cultures were subsequently discarded while 9, chosen at random, were identified as Ps. fluorescens or variants. Young litmus milk cultures of these organisms yielded odors which were difficult to distinguish from the odor of a similar culture of Ach. putrefaciens. However, the technique of spreading the culture on the fingers and smelling easily differentiated the two organisms on an odor producing basis. Older cultures were distinctly "cowy" in odor somewhat suggestive of rancidity but bearing no resemblance to either surface taint or "sweaty-feet" odor.

Two litres of a 2 week old skimmilk culture of Ps. fluorescens were steam distilled according to the methods of Dunkley (1940) and the distillate caught in $Ba(OH)_2$ solution. Fatty acid rather than "sweaty-feet" odor was noticed from the acidified residue obtained from the evaporation of the distillate.

Portions of aseptically-drawn milk were heat treated as mentioned under a preceding heading. These milks were inoculated with Ps. fluorescens and incubated at room temperature. At various intervals they were tested for odor. All samples emitted a strong rancid odor, which at no time suggested surface taint or "sweaty-feet".

Churning experiments involving seven cultures chosen at random were performed. Seventeen churnings using pasteurized cream were made, two of which were salted. Three churnings using raw cream were made, two of which were salted. Of the 15 pasteurized, unsalted butters 8 were graded surface taint or questionable surface taint 24 hours after churning. The eight churnings involved four separate bacterial cultures. None of the raw-cream butters showed even questionable surface taint. Forty-eight hours after churning all the unsalted butters were unquestionably rancid. Salt appeared to have an inhibitory effect on the development of rancidity, for it developed later in those butters containing salt. It is evident that incipient rancidity in butter is sometimes difficult to distinguish by smell from typical surface taint. However, it is well to mention here that the results obtained with this organism are by no means as clear-cut, as between raw and pasteurized cream butter, as the above description would indicate.

The results of these experiments with Ps. fluorescens lack the clarity of similar trials with Ach. putrefaciens. There is some indication that for very short periods commercial butter might be graded surface taint because of the effect of Ps. fluorescens, but they would undoubtedly very quickly develop rancidity. Organisms of this type are almost invariably present in Alberta butters, oftentimes in comparatively large numbers even in normal butters. If they are responsible for commercial surface taint there is no apparent explanation for the sudden appearance of surface taint in Alberta butters about 1919 and its frequent occurrence since. Practical butter men report that frequently surface taint butters later develop rancidity. In view of the widespread distribution of organisms of the Ps. flourescens type and their relation to rancidity it is impossible to say at the present time whether rancidity is part of the surface taint problem.

XIV. THE CONTROL OF SURFACE TAINT

Since surface taint is undoubtedly bacterially produced the methods for its control rest, logically, in the elimination of the bacteria themselves. Both experience and experiment suggest that water used for washing and rinsing plant equipment may introduce the bacteria into the creamery. Adequate water treatment for country creameries is a major problem itself. Of the methods available, chlorine treatment may ultimately prove to be the most economical, but for consistent results the process must be carried out with minimum chances for slip-ups. Failing the use of water treatment it would seem that rigorous plant

sanitation would be the means of removing from equipment not only the bacteria but also milk solids upon which the bacteria may grow to produce the substance(s) which subsequently is incorporated in the butter.

The basis for the use of these measures may not be as theoretical as might be inferred. Two instances are at hand where the institution of such methods seems to have brought surface taint entirely under control. In Plant 'A' the amount of surface taint butter approached 20% of the total make during a period of approximately 2 months in which it came under our observation. The next year, when chlorine treatment of all water coupled with rigorous plant sanitation was instituted, surface taint butter was no longer produced. It seems logical to hold these measures responsible for the disappearance of surface taint butter from this plant. Plant 'B' had a similar history, although the initial incidence of surface taint was not as high as in Plant 'A'. Measures similar to those adopted in Plant 'A' resulted in complete abeyance of the difficulty.

XV. SUMMARY AND CONCLUSIONS

1. Surface taint butter seems to have quite a wide geographical distribution.

2. Surface taint butters fell within the range of normal commercial butters for the following values:

1. Salt content.
2. Curd content.
3. pH.
4. Total and proteolytic counts on two media incubated at 10-15°C up to 5 days.

3. Surface taint production in butter, incubated at 10-15°C, by Ach. putrefaciens grown in high-temperature pasteurized cream prior to churning has been confirmed.

4. Ach. putrefaciens or variants were isolated from 2 samples of surface taint butter, from 3 samples of normal commercial butter, from 12 samples of water.

5. Ach. putrefaciens is probably water-borne and appears to be quite widely distributed throughout the province of Alberta.

6. Storage at around 0°F does not appear to be a satisfactory method for improving the grade of surface taint butter.

7. Due to a demonstrated difficulty in differentiating ordinary putrefactive and surface taint odors, the validity of the seemingly high incidence of surface taint in unworked and melted butters is questioned. It is believed that the growth of many bacterial types in the free moisture was responsible for the production of odors difficult to tell from surface taint.

8. In a study of Ach. putrefaciens the following features were noted:

(a) It appears to have quite strong gram negative staining reactions and characteristics.

(b) The pH range of growth of the organism extended from 5.26 to 8.20 in broth or peptone water and it grew at a pH of 9.50 in skimmilk. A putrid rather than the "sweaty-feet" odor was evident in a skimmilk culture of Ach. putrefaciens having a pH of 7.6 or higher. Acidification of the culture released the "sweaty-feet" odor. The production of a putrid odor along with

the "sweaty-feet" odor was demonstrated by aerating a skimmilk culture of Ach. putrefaciens.

(c) Growth of Ach. putrefaciens in broth was inhibited by over 6% NaCl and in skimmilk by over 4%. Surface taint production by Ach. putrefaciens was not inhibited in butter containing 2.5% added salt. This finding is in accord with those of Hood and White (1928) and others.

(d) Ach. putrefaciens has reducing properties in broth, glucose broth and skimmilk as shown by E_h measurements by the electrometric method. The anti-oxidant properties of Avenex or hydroquinone in butter made from cream inoculated with Ach. putrefaciens may account for the inhibition of surface taint.

(e) Ach. putrefaciens has marked proteolytic properties in skimmilk culture as shown by soluble nitrogen determinations. The organism apparently is able to utilize inorganic as well as organic sources of nitrogen in its metabolism and therefore may eventually be classed in Family Pseudomonadaceae (Bergey).

(f) Ach. putrefaciens did not produce H_2S from skimmilk or nutrient broth unless cystine were added. H_2S was produced in nutrient broth containing various amounts of skimmilk powder. H_2S and "sweaty-feet" odor production tended not to be produced simultaneously.

(g) "Sweaty-feet" odor was not produced in raw milk, skimmilk or cream nor in these products heated to 145°F for 10 minutes but was produced when the heat-treatment was 145°F for 30 minutes or at higher temperatures for 10 minutes.

Surface taint production by Ach. putrefaciens was not noticed in raw-cream butter.

9. Surface taint was not noticed in inoculated cream which had been repasteurized before churning.

10. Surface taint was produced when butter was worked on a damp surface contaminated by previously working an inoculated-cream butter. Ach. putrefaciens was easily isolated by the plating technique from such butter.

11. Surface taint was produced by washing butter with water containing Ach. putrefaciens, which was isolated from those butters showing the defect.

12. The "sweaty-feet" odor was very questionably produced in nutrient broth or peptone water. At times no odor whatsoever was noticed. Broth cultures of Ach. putrefaciens in the presence of various substances like proteins or derivatives, amino acids or fatty acids failed to show the "sweaty-feet" odor.

13. The "sweaty-feet" odor was produced in butter moisture provided the salt content were low enough to allow growth.

14. The magnitude of the proteolytic count of butter in T.G.S. agar may not reflect the presence of Ach. putrefaciens as shown by picking colonies into litmus milk.

15. Experimental butters stored at 10-15°C sustained variable increases in total and proteolytic counts on T.G.S. agar.

16. Ps. fluorescens does not produce surface taint or "sweaty-feet" odor in milk, regardless of previous heat treatment.

17. The odor produced by Ps. fluorescens in several, but not all, experimental pasteurized-cream butters incubated at 10-15°C

for 24 hours following churning was indistinguishable from surface taint. Longer incubation, however, resulted in rancidity, which was somewhat inhibited by salt in the butter. The results with this organism on the production of surface taint in raw- and pasteurized-cream butters lack the clarity of those with Ach. putrefaciens.

18. A general suggestion for the control of surface taint in butter is made.

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